CYTOLOGICAL GRADING OF DUCTAL CARCINOMA OF BREAST IN FINE NEEDLE ASPIRATES AND ITS CORRELATION WITH HISTOLOGICAL GRADING

Dissertation submitted in

Partial fulfillment of the regulations required for the award of

M.D. DEGREE

In

PATHOLOGY – BRANCH III

THE TAMILNADU

DR. M.G.R. MEDICAL UNIVERSITY

CHENNAI

APRIL, 2013

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I hereby declare that the dissertation entitled "**CYTOLOGICAL GRADING OF DUCTAL CARCINOMA OF BREAST IN FINE NEEDLE ASPIRATES AND ITS CORRELATION WITH HISTOLOGICAL GRADING"** was done by me in the Department of Pathology at Coimbatore medical college, Coimbatore during the period from August 2011 to July 2012 under the guidance and supervision of **Dr.C.Lalitha.M.D.,** Additional Professor, Department of Pathology, Coimbatore medical college, Coimbatore. This dissertation is submitted to the Tamilnadu Dr.M.G.R.Medical University, Chennai towards the partial fulfillment of the requirement for the award of M.D., Degree in Pathology. I have not submitted this dissertation on any previous occasion to any university for the award of any degree

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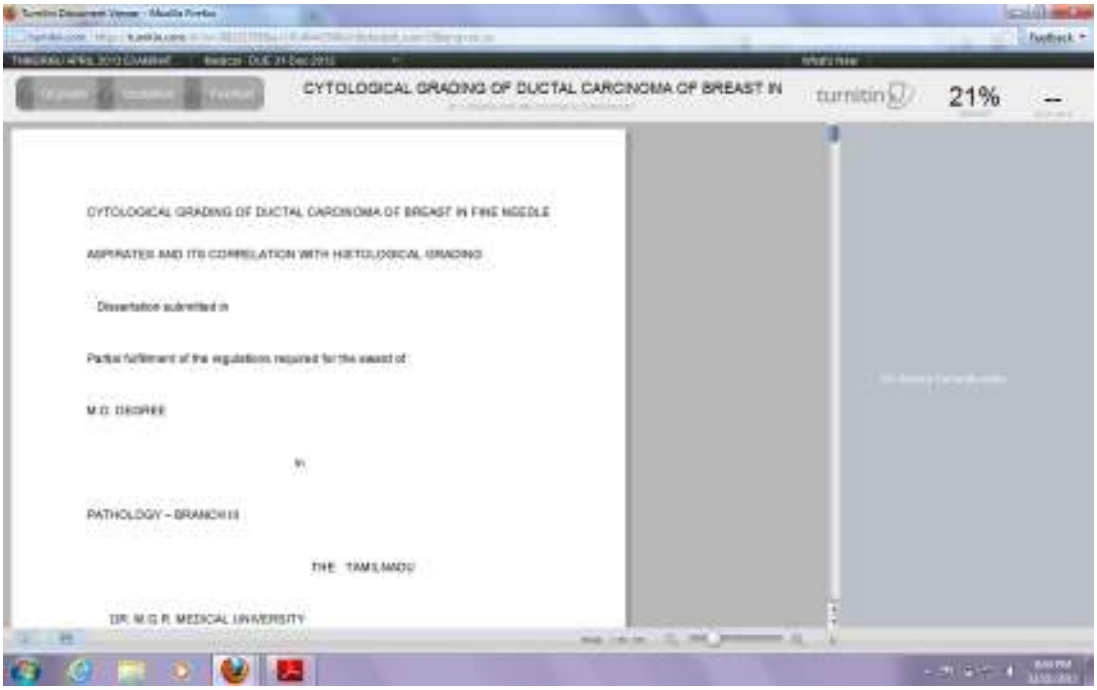

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DECLARATION I
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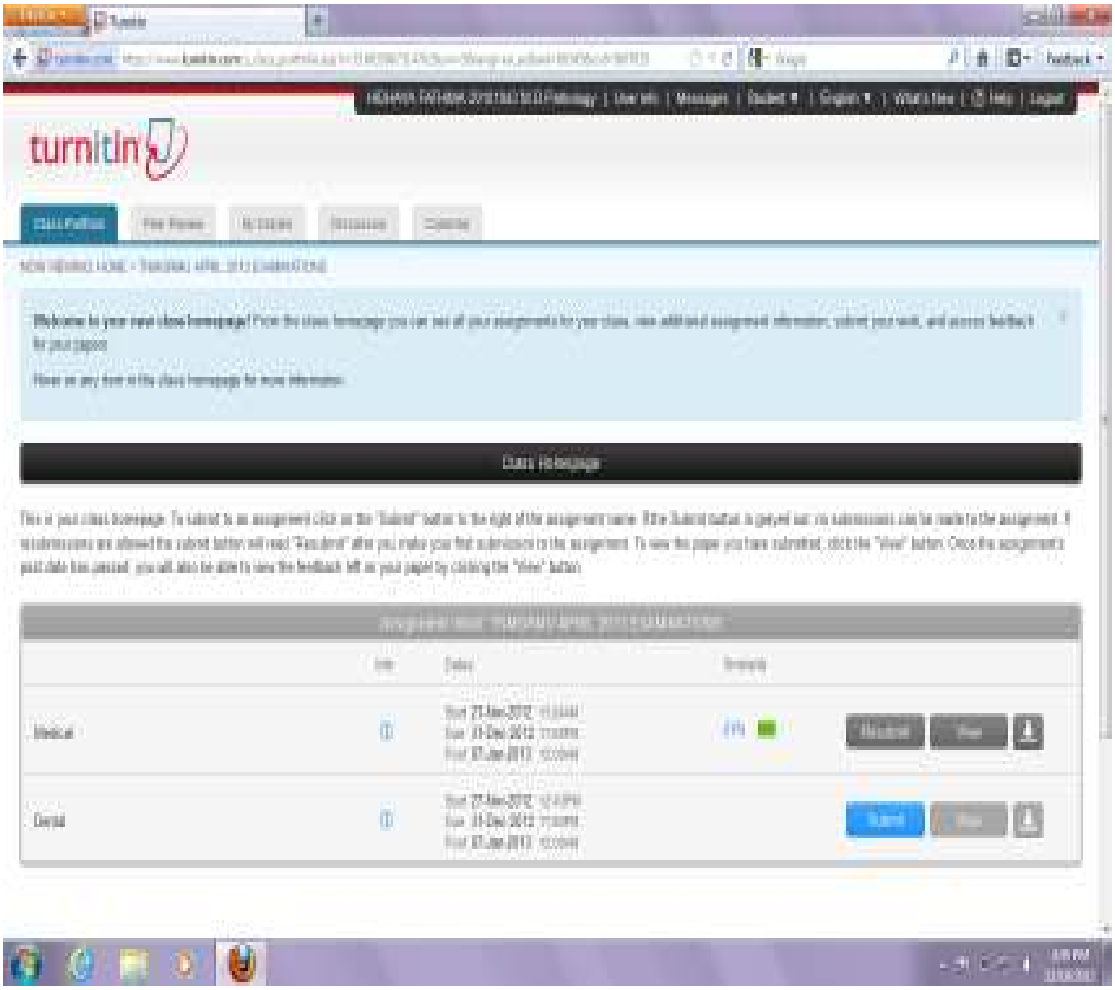
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ABBREVIATIONS

DC-Ductal carcinoma

IDC NOS-Invasive duct carcinoma no special type

FNAC -Fine needle aspiration cytology

NCI -National cancer institute

NCHG-Nottingham combined histologic grading

H&E- Haematoxylin and eosin

ICMR- Indian council of medical research

TDLU-Terminal duct lobular unit

WHO-World Health Organisation

DCIS-Ductal carcinoma in situ

Pap stain-Papanicolaou stain

CG-Cytologic Grade

HG-Histologic grade

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INTRODUCTION

Breast cancer is the second most common cancer in females world wide accounting for about 1.4 million cases each year, with more than half of the deaths due to breast cancer occurring in low and middle income countries. Breast cancer is the second leading cause of cancer related deaths in females all over the world[66]. Breast cancer have doubled in India in the last two decades.

The number of women estimated to be dying of breast cancer every year has also been steadily raising. As against an estimated 48,170 women who died of breast cancer in 2007, the number reached the 50,000 in 2010,which raised to 50,821 by 2011.An ICMR study in many cosmopolitan cities like Chennai revealed that the incidence of cervical cancer had been dropped and breast cancer incidence were emerging. Between 2006-2008 the incidence of breast cancer in Chennai was 26.5%(ICMR).As per study conducted in Coimbatore, percentage of occurrence of breast cancer was found to be 20.3out of 1 lakh women.

Fine needle aspiration cytology is being increasingly used to determine the benign and malignant nature of the lesion. This also provides additional information about the intrinsic nature of the lesion and the prognosis[12].Attempts have been made to determine various prognostic parameters on FNA Material to determine the best therapy in a

given case [68,69]. Due to its tremendous increase in incidence rates, it is desirable to grade the tumor while is still in vivo for the selection of appropriate therapy and to avoid overtreatment for low grade carcinoma.

The significance of histological grading was accepted .It was also found to be a useful supplementary prognostic parameter .So when a significant association was established between cytologic and histologic grading systems, cytologic grading may be helpful to decide the level of treatment.

AIM OF THE STUDY

To evaluate the cytological grading by Robinson method in FNAC samples and to correlate it with the well known prognostic factor, Histological grading system proposed by Nottingham Modification of Bloom Richardson .The cytologic grading system was also correlated with the axillary lymph node status.

OBJECTIVE

1. To grade the cytological features of the fine needle aspirates of ductal carcinoma of breast
2. To grade the histomorphological features of invasive duct carcinoma in mastectomy specimens
3. Correlate statistically the cytological grading system with histological grading system.

REVIEW OF LITERATURE

Breast carcinoma is the second most common malignant tumor in females, with more than 100,000 cases occurring worldwide annually[2]. As per datas given by the World Health Organization there are 7.6 million cancer deaths per year all over the world, out of which 5,02,000 are due to breast cancer alone. With such an increased rate, breast cancer is one of the most fatal cancers. The incidence is high in North America and North Europe (91.4 new cases per 100,000 women / year) intermediate in southern and latin American countries and low in Asian and African countries[2].It can occur at any age, but the peak incidence is 45-60 years. The frequency is increasing in younger age groups and this is not due entirely to an increase in "at risk" population. Breast carcinoma is 20 times more common in women than men. Affected males tend to be somewhat older.

Wani et al reported that, In India breast is reported as the most common site of cancer in Mumbai and Thiruvanandapuram, second most common site in Chennai and dibrugarg[18]. The most common tools used for diagnosis are mammography and a fine needle aspiration biopsy (FNA). Mammography is a non-invasive method, used for screening purposes rather than for precise diagnosis.A fine needle aspiration biopsy

is an invasive method to obtain a small sample of the breast tissue that allows the pathologist to diagnose the type of the cancer in detail.

ANATOMY OF BREAST:

The adult mammary gland is a compound tubulo -alveolar gland. The mammary gland is covered with skin and subcutaneous tissue and rests on pectoralis muscle, from which it is separated by a fascia.

The functional unit of breast is composed of two major parts: Terminal duct lobular unit (TDLU) and larger duct system. The TDLU is formed by the lobule and terminal ductule and represents the secretory portion of the duct.

HISTOLOGY OF BREAST:

A Glandular lobule consists of intralobular ducts surrounded by loose intralobular connective tissue that contains fibroblast, lymphocytes and plasma cells. Intralobular ducts are lined by a specialized two layered epithelial cells; cuboidal to low columnar epithelial cells and basal myoepithelial cells. Lobule is surrounded by dense interlobular connective tissue which contains a large interlobular duct.

CYTOLOGY IN LESIONS OF BREAST:

Fine needle aspiration cytology is a valuable tool in the work up of all breast abnormalities, both palpable and non palpable. Fine needle aspiration cytology of breast was first used in 1930 by Mortan Ellis and Stewart. It was increasingly used for the preoperative diagnosis of breast carcinoma[4].

FNAC has a sensitivity of 87%, specificity of 99%, negative predictive value of 99%[7,8,9]. The false negative rate of only 10% has been reported [8,9]. The percentage of inadequate specimens should be always less than 10%[10].The false positives are always interpretation errors. The diagnostic accuracy of FNAC is defined as a correlation with histologic findings, depending on the availability of a good aspirate and good abilities of reporting[14]. One of the major drawbacks of this technique is obtaining an insufficient specimen.

The NCI guidelines recommend the use of 22-23 gauge needle aimed at the central portion of the tumor and moved at different directions. For fibrotic and necrotic lesions needle should be aimed at the periphery of the tumor. There were no strict requirements for the number of ductal epithelial cells to be present for sample to be adequate. Adequacy can be determined by the aspirator and the pathologist who examine the slide.

The NCI conference proposed to classify the cytological features into the following categories:

- benign
- atypical/indeterminate
- suspicious/indeterminate
- suspicious/probably malignant
- malignant
- unsatisfactory

The guidelines emphasizes to include the microscopic findings that are specific for each category.

The FNAC procedure induce histologic changes of the lesions which includes extensive necrosis, epithelial displacement and hemorrhage with or without organization. Needle tract seedling has not been reported with breast cancer. The advantage of FNAC in breast lesions lies in the precise diagnosis, minimal or no morbidity and good patient acceptance.

INVASIVE DUCT CARCINOMA :

Invasive duct carcinoma is the group of malignant tumors of the breast which have the tendency to invade the adjacent normal breast tissues and also the distant organs. They can be classified into various

histological types. Invasive duct carcinoma Not otherwise specified (NOS) is the most common type. Various terms have been used to describe such tumors, including Ductal carcinoma, Carcinoma not otherwise specified and invasive carcinoma of no special type. For the present time, the preferred nomenclature accepted by WHO is Invasive duct carcinoma ,no special type .This comprises about 75-80% of breast carcinoma[25,26].This also includes tumors that express in part one or more characteristics of the specific types of breast carcinoma but do not constitute the pure examples of the individual tumors.

The relatively favourable prognosis associated with some special types has been found to apply only to those tumors composed entirely or large proportion by the specific pattern .When these features are less extensively represented, the prognosis depends on the broader group of Invasive duct carcinoma NOS. Tumors combining features of invasive duct carcinoma with pagets disease are classified as Invasive duct carcinoma. Approximately one third of the lesions characterized as Invasive duct carcinoma NOS has one or more of the combined features. More than half of the combined tumors were ductal carcinoma with tubular carcinoma component. Combinations with invasive lobular carcinoma were detected in 6% of lesions. There is a significant correlation between the grade of the intraductal and invasive component

in those tumors that have both components.This indicate that the prognosis of the tumor can be assessed in the preinvasive stage itself. The tumor can arise anywhere in the breast, the most common site being outer and upper quadrant.

The main presenting complaint in breast carcinoma is palpable breast lump. They vary in size from one to several centimeters in diameter, are firm to hard and the edge may be well or poorly defined. About 2 percent of the patients present with the synchronous bilateral tumors. Nipple discharge is an infrequent presenting sign and the breast pain is very rarely a manifestation of malignancy. In the recent days, the introduction of mammographic breast screening has changed the pattern of presentation .In the target age group many cases are detected because of mammographic screening which detects impalpable mammographic abnormality

FNAC in most of the Invasive duct carcinoma gives a high yield than the normal breast tissue.The only exception was schirrous carcinoma where it gives a scanty aspirate.Morphologic classification of breast cancer was also done in FNAC specimens. Based on the cytomorphological criteria proposed by World Health Organization , 254 various histologic types of invasive breast carcinoma can be diagnosed . This morphological classification has some impact on the prognosis.

Certain subtypes has favourable prognosis which includes pure mucinous (colloid), true medullary, tubular carcinoma, adenoid cystic carcinoma, papillary carcinoma, and secretory carcinoma whereas certain subtypes have unfavourable prognosis which includes metaplastic carcinoma, inflammatory carcinoma, pleomorphic lobular carcinoma.

As per the American Cancer Society, about two-thirds of women with an invasive duct carcinoma NOS are 55years or older. The overall survival rate is 55-65%.

DIAGNOSTIC CRITERIA FOR DUCT CARCINOMA IN CYTOLOGY:

- Cellular smear
- Malignant epithelial cells arranged in three-dimensional clusters, syncytium, and acini
- Single population of cells
- No myoepithelial cells; no bare or bipolar nuclei
- Moderate to severe nuclear atypia
- Uneven necrosis
- Tumor diathesis may be present
- Some cases demonstrate cytolpasmic vacuolation or intracellular lumina

CRITERIA SUGGESTIVE OF INVASIVE LESION IN CYTOLOGY:

- Proliferation of fibroblast
- Cell poor elastoid stromal fragments
- Single cells or small clusters of cells in stromal tissue and fatty fragments
- Tubular structures
- Intracytoplasmic vacuoles

HISTOPATHOLOGIC FEATURE IN INVASIVE DUCT CARCINOMA NOS:

Invasive duct carcinoma NOS is a diagnosis of exclusion and there are no specific morphological features and a variety of histological appearance may be seen. These include tumors which do not satisfy the criteria necessary to qualify for any other special types

Gross: Firm to hard, poorly circumscribed, gritty to cut, trabaculae extending to surrounding parenchyma. The size may vary from 0.5-10cm

Microscopy: Tumor arranged in diffuse sheets, well defined nest, or as individual cells, nuclei may be regular or large pleomorphic with prominent nucleoli.Mitotic figures may be seen depending on the grade

of the tumor. The stroma may be densely fibrotic to highly cellular. To be typed as Invasive duct carcinoma NOS, 90% must be composed of that type. If the Invasive duct NOS comprises between 50-90%,then it will be classified as mixed type. In most cases areas of associated DCIS and occasionally areas of squamous metaplasia may be seen. Some shows foci of neuroendocrine differentiation.

OTHER SPECIAL TYPES:

TUBULAR CARCINOMA:

The frequency of tubular carcinoma is 1-3 %.Tubular carcinoma is widely recognized to have an excellent prognosis.

Cytology: Low to moderately cellular smears, predominately in cohesive clusters, angulated rigid open tubules and glands with comma shaped projections. Mild to moderate nuclear atypia made out. This is one of the tumor which is under diagnosed due to the relatively monomorphic appearence of the tumor cells with mild atypia.

Gross: Pure tubular carcinoma is relatively small, usually measures between 2mm and 1.5cm in diameter, most of them are less than 1 cm in diameter. Two morphologic subtypes are encountered they include “pure”

subtype in which stellate nature is pronounced and “sclerosing” subtype where it is diffuse and more ill defined

Histopathology: Well differentiated neoplasm with irregular angulated tubules with open lumina lined by only one layer of epithelial cells with mild pleomorphism. The stroma is abundant and cellular.The nucleus show little pleomorphism with rare mitoses. For a tumor to be typed as tubular carcinoma , tubular morphology should comprise >90% of its area. If less than 90%, it is classified as the mixed type. But for the combination of tubular and cribriform carcinoma, that tumor is classified as tubular, if the tubular pattern comprises > 50% of the tumor area.

MUCINOUS CARCINOMA:

The frequency of the tumor ranges from 1-4%.It has a better survival rate compared to the usual infiltrating ductal carcinoma of breast. This type is also referred to as colloid carcinoma. These are tumors in which mucin production is apparent to naked eye. These comprises only tumors entirely composed of mucinous patern, owing to its favorable prognosis.

Cytology: Aspirate yields a gelatinous material. Atypical cells arranged in solid clusters or single files with moderate degree of nuclear atypia in a background of abundant mucin.

Gross: Well circumscribed tumor mass with a soft consistency and a glistening gelatinous cut surface.The size may vary from 1-4cm

Histopathology: Small clusters of regular epithelial cells floating in a pool of extracellular mucin .The cells are small with darkly staining nuclei which exhibiting mild pleomorphism. Mitoses are infrequent.

MEDULLARY CARCINOMA:

The reported frequency varies between 2-10 percent.

Cytology: Highly cellular smears with poorly cohesive cells, large pleomorphic nuclei and many lymphocytes and occasional plasma cells. The false negatives can be reduced by careful search of malignant epithelial cells in the aspirates with increased number of lymphocytes in the background .

Gross: Well circumscribed tumor with pushing margins, with a soft and uniform consistency, usually measuring between 1-4 cm

Histopathology:

Three main morphologic criteria for diagnosis of medullary carcinoma

1. Epithelial cells arranged in interconnecting sheets forming a syncytial network. Syncytial pattern of growth comprise >75% of tumor. Tumor cells are large pleomorphic with prominent nucleoli and high mitotic rate.
2.The intervening stroma contains lymphoplasmacytic infiltrate.

3. Grossly evident circumscription, border of the tumor is pushing rather than infiltrative.

ATYPICAL MEDULLARY CARCINOMA:

Features of typical medullary carcinoma but no more than two atypical features. The atypical features include infiltrating margins, absent or mild lymphoplasmacytic infiltration, uniform nuclei, focal tubule formation and presence of in situ carcinoma.

MICROPAPILLARY CARCINOMA:

Invasive micropapillary carcinoma is a recently diagnosed variant of Invasive duct carcinoma

Cytology:

- Cellular smears
- Cells arranged in multilayered sheets, rounded aggregates with distinct anatomical borders without a stromal core.
- Individual cells are cuboidal to columnar with moderate nuclear atypia.

Histopathology: Cluster of epithelial cells arranged in a micropapillary or tubular-alveolar pattern without a fibrovascular core suspended in a clear space. Cell clusters appear to have an inside out appearance. This tumor has an increased incidence of vascular invasion and lymph node involvement .It has a poor prognosis.

PAPILLARY CARCINOMA:

It constitutes <1% of symptomatic cases. It is frequent in elderly.

Cytology:

- Highly cellular smear
- Complex branching papillae with thin fibrovascular core
- Papillae lined by multilayered epithelial cells
- Marked discohesion

- Tall columnar cells
- Malignant epithelial cells with nuclear atypia and enlargement
- No myoepithelial cells
- Background shows blood and hemosiderin-laden macrophages

Gross: Most of the tumors are well demarcated, soft in consistency , measure between 1-3 cm.

Histopathology:Presence of papillary structures with fibrovascular cores. Cytologic appearance may be varied. Nuclear pleomorphism and increased mitosis may be seen.

SECRETORY CARCINOMA :

These tumors are described in children, but are now known to occur in adults of all ages. Secretory carcinoma of the breast was first described by McDivitt and Stewart in 1966.It is one of the rarest tumors.

Cytology: Loose aggregates of malignant epithelial cells with abundant granular, eosinophilic vacuolated cytoplasm and a relatively bland nuclei.

Gross: Well circumscribed tumor, usually less than 2 cm in diameter.

Histopathology: Tumor islands of irregular tubules containing eosinophilic material. Cells have abundant pale staining cytoplasm with nucleus showing mild pleomorphism. Tumor shows a well circumscribed border with peripheral fibrosis. The diagnostic feature is the presence of intra and extracellular rounded vacuoles giving a overall clear cell pattern.The individual cells show mild nuclear pleomorphism with infrequent mitoses.These vacuoles stain positive for Alcian blue and PAS with diastase resistant.

APOCRINE CARCINOMA:

Invasive duct carcinoma show focal areas of apocrine metaplasia. But tumors composed entirely of apocrine cells are less frequent.The prognosis of these tumors were similar to that of invasive duct carcinoma NOS.

METAPLASTIC CARCINOMA:

Metaplastic carcinoma is characterized by a mixture of ductal carcinoma with areas of spindle, squamous, chondroid, or osseous metaplasia

Cytology: Tumor with malignant ductal cells and spindle cells. Multinucleate giant cells may be seen. The sarcomatoid component is usually malignant spindle cells and in some cases it may show osteosarcomatous or chondrosarcomatous differentiation

Gross: Large, firm nodular tumors usually more than 5cm in diameter. Tumor is fixed to deep fascia.

Histopathology: Malignant ductal carcinoma which in addition to epithelial elements show transtition to sarcomatous elements including cartilage, bone, myxoid stroma and spindle cell stroma. The most common metaplastic component is malignant spindle cells.There are two types: monophasic sarcomatoid and biphasic sarcomatoid. More than one sarcomatous component can be seen. It is difficult to assess the prognosis of metaplastic carcinoma because of its rarity, but available evidence suggests that these tumors behave as a highly malignant tumors with early recurrence and poor survival.

CARCINOMA WITH NEUROENDOCRINE DIFFERENTIATION:

Cytology: Cell rich smears of numerous dispersed small uniform cells with a coarse granular chromatin.

Histopathology: Small tumor cells arranged in solid nest separated by fibrous stroma. Tumors with neuroendocrine differentiation is found to have an improved prognosis

LOBULAR CARCINOMA:

The classical form of lobular carcinoma was first described by Foote and Stewart.

Cytology: Smears have a scant cellularity. Cells arranged in small clusters and single files .Individual cells are small round uniform with hyperchromatic nuclei and scant cytoplasm. Intracytoplasmic lumina, mucin vacuoles, nuclear moulding and signet ring cells may be present, No bipolar nuclei are seen in the background.

Histopathology: Classic variant: Small uniform cells growing in a single file and in a concentric fashion around the lobules. The stroma is abundant and fibrous. The individual cells are small round to ovoid with little cytoplasm with eccentrically placed nucleus exhibiting mild pleomorphism and infrequent mitoses.

Other variants include:

Alveolar: Cells similar to classic type arranged in aggregates of 20 or more cells.

Solid: Cells infiltrate diffusely in sheets rather than cords with little intervening stroma.

Tubulolobular: Cells arranged in cords and tubules. These tubules are much smaller than that of tubular carcinoma.

Pleomorphic: Classical lobular infiltrative pattern with cells exhibiting cellular atypia and nuclear pleomorphism. These cells have more eosinophilic cytoplasm.

MIXED TYPES:

The mixed ductal and lobular comprises of tumors in which the ductal component constitutes between 10 and 90 percent of the tumor. The mixed ductal and special type include any tumors composed of mixture of tubule-lobular, tubular,cribriform or mucinous carcinoma with ductal NOS in which the later forms over 10 percent of the tumor.

PROGNOSTIC FACTORS ON CYTOLOGIC SAMPLES OF DUCTAL CARCINOMA OF BREAST:

In recent years attempts have been made to determine the various prognostic parameters on FNA material for management. Evaluation of prognostic factors is helpful in the decision of preoperative neoadjuvant chemotherapy in high grade tumors, avoidance of conservative surgery in high grade tumors and usage of specific treatment modalities like Tamoxifen ,herceptin. The various prognostic factors that can be evaluated in cytological samples include tumor grade, estrogen receptor status and proliferative activity

Jayaram et al[11] states that of the various prognostic factors, tumor type, tumor grade, receptor status and cellular proliferative rate are the important prognostic factors that can be validated at cytological level. Among these tumor grade is the easy, feasible, assessable and cost effective prognostic factor [11]

CYTOOGICAL GRADING SYSTEM IN DUCTAL CARCINOMA OF BREAST:

The National cancer Institute (NCI) Bethesda, sponsored conference had also recommended that tumor grading on FNA material

should be incorporated in FNA reports for prognostication[13.] It was emphasized that grading system used in cytological samples should correspond to the grading system used in histological specimen.

Wani et al[18] had stated that grading of breast carcinoma, when the tumor is still in vivo, would be the best and desirable method, because it would be helpful for the selection of appropriate therapy.Preoperative cytological grading will give an indication for presumed aggressiveness rather than therapeutic implication. Various grading systems were introduced in cytological samples. They include Black's nuclear grading system which is a two tier grading system, which differentiates into low grade and high grade carcinoma[36,37.]

Then the Fishers modification of Blacks grading system which is a three tier grading system which grades into well differentiated, moderately differentiated and poorly differentiated grade[15,16]. Howell et al. attempted to grade the cytological features of breast carcinoma using Elston and Ellis-modification of the Scarff–Bloom–Richardson grading system .But this attempt is of little success due to difficulties in assessing tubule formation and counting mitosis in cytologic smears.

The other grading system used were Masood cytological grading system and Hunt's grading system. Of the various grading systems, Robinson and Mouriquand corresponds to the histological grading by

the Nottingham modification of Scarff Bloom Richardson method .The diagnostic accuracy and sensitivity of both Robinson and Moriquand grading systems were similar.However the specificity of Moriquand system was quite low[17]. However Robinson grading system has been found to be easier and better because of more objective set of criteria and reproducibility [18].In a study by Bhargawa et al comparing Robinsons' method of cytological grading, Fischer modification of Black's nuclear grading, Scarff bloom Richardson grading on cytological samples, Robinson method showed a good correlation with histological grading.

In a study by Rekha et al, various special types like colloid carcinoma, tubular carcinoma and lobular carcinoma and medullary carcinoma were also graded using cytological grading system by Robinson method[19.]A study by Khan MZ et al have also graded certain special types of ductal carcinoma which includes tubular, medullary, papillary and lobular carcinoma. Because nuclear grading does not involve an assessment of the growth pattern of the tumor, it applies to other subtypes of breast carcinoma also.

The Robinson method of cytological grading system include the following factors: Cell dissociation, cell uniformity, nuclear size, chromatin pattern, nucleoli, nuclear membrane. Among various factors cell dissociation, chromatin pattern and presence of nucleoli were the most significant factors[20]. They also found that cell uniformity and

nuclear margins corresponds to the nuclear pleomorphism in histologic grading .So these parameters are given more importance since tubule formation and mitotic rate were difficult to analyze in cytological samples. This is the reason why Scarff Bloom Richardson grading system cannot be applied on cytologic samples. Nuclear grading can be performed in May Grunwald Geimsa, papanicolaou and H&E stained smears. In a study by Idris et al papanicolaou stain showed the good quality of staining with a high quality index of 0.87, next ranked the H& E stain with a quality index of 0.81, followed by MGG stain with a low quality index[21].Pap stain is advantageous over other stains as it stains the nuclear chromatin well and gives a good differential cytoplasmic counterstaining.

PROGNOSIS IN INVASIVE DUCTAL CARCINOMA OF BREAST

Breast carcinoma is a heterogeneous disease clinically, pathologically, radiologically and differs in its biological potential. As noted by Sistrunk and Mc carty, It is impossible to foster the duration of life of patients with carcinoma of the breast, because the degree of malignancy varies widely and patients react differently to the disease[22]. In a report by Heilmann and Helmann[22], risk of recurrence and time of recurrence depends on tumor size and nodal status[23].

In a similar type of analysis by Blamy et al[24], tumor grade was assessed with risk and time of recurrence. They observed that 90% recurrence occurred within 9,7and 5 years with grade 1,2and 3[24].The rate of death due to breast carcinoma was also influenced by tumor grade with 90% occuring in 40,13 and 8 years in grade 1,2and 3 tumors. The study by Blamey et al also states that histologic grade proved to be an additional determinant of metastagenicity and virulence of tumors.

TUMOR SIZE:

For prognostic purposes the size of the tumor should be evaluated by the pathologist, as clinical measurement is not sufficiently required. The measured gross size, which is represented by the largest diameter of the tumor is one of the most significant variable. Tumor size and lymph node status are two independent prognostic variables[27,28,29]. Datas from the breast cancer surveillance consotorium for 786,846 women ages 40-89 years with breast carcinomas revealed negative axillary node metastasis in 91.8%,78.2%,57.9% of patients with tumor size measured 0-10mm,11-20 mm,21-50mm respectively [30].Queit et al states that the disease free survival after mastectomy was 81% in tumors <2cm and 59% in tumors >2cm [31].Tumors less than 10 mm in diameter are called the minimal invasive carcinoma, which represent the earlier stage of the disease.

Recommendations for measuring the tumor size:

1. Tumor should be measured in all three dimensions to the nearest millimeter before fixation. The measurement are checked after fixation and the single largest dimension should be taken into account.

2. For tumors less than 10mm and for cases in which there is large in situ component, microscopic assessment of tumor size using stage micrometer is accurate.

3. When both in situ and invasive components are seen, measurement of invasive component is taken into account, in such cases microscopic assessment of tumor size is more accurate.

4. For multifocal and multicentric lesions are seen, size of the largest invasive lesion is considered

In Nottingham/Tenovus study, tumor <15 mm in size confer a good prognosis with favourable clinical outcome[2].So the cut off point for long term survival is 15mm. Furthermore the Nottingham /Tenovus Primary Breast Cancer Study has confirmed by multivariate analysis that tumor size is an significant independent variable forming an integral component of Nottingham Prognostic index[33,34]

LYMPH NODE STATUS:

Axillary lymph node status is an important indicator of systemic adjuvant therapy.It is based on the histological examination of excised nodes rather than clinical examination. Nodal metastasis are divided into three categories based on size .Numerous studies have shown that patients with positive loco-regional nodes have poor prognosis when compared to those without nodal involvement[33,35].

Prognosis also depends on the number and level of regional nodes involved..The greater the number of nodes involved poorer the prognosis[36,37] .Nodes in the higher or more distal levels of axilla carries a worse prognosis[38,39].High axillary clearance usually shows increased morbidity. The introduction of sentinel node biopsy with or without additional node sampling resulted in reduced use of axillary clearance. Low axillary clearance ,below the intercostal nerve produces sufficient lymph nodes (4-15) for accurate prognstication .

Lymph node metastasis is divided into three categories based on the size

>2 mm- macrometastasis

0.2-2mm-micrometastasis

<0.2mm- isolated tumor cell and tumor cell clusters

Lymph node stage is divided into three categories as follows

PN1-1-3 nodes

PN2-4-9 nodes

PN3->10 nodes

Recommendation for analysis of lymph node status :

1. Every lymph node obtained should be examined histologically.
2. Grossly uninvolved nodes are submitted as a whole where as only representative sections of the involved nodes are submitted.
3. Histopathology report should include total number of enlarged lymph nodes, number of positive lymph nodes and the greatest dimension of largest metastatic node and extranodal extension.
4. Lymph nodes under 5mm can be bisected and blocked.
5. Lymph nodes >5mm should be sliced at 2-3 mm intervals perpendicular to long axis.

Jean. F.Simpson states that even though lymph node is the most important predictor of prognosis, lymphnode status alone is not sufficient for treatment decision to be made[40]

TUMOR CONFIGURATION:

Tumors with circumscribed margins have a better prognosis than tumors with infiltrative margins. Infiltrative tumors tend to be larger and likely to have axillary node metastasis[41,42].Tumors with stellate configuration with focal necrosis have poor prognosis [42]

HISTOLOGIC TYPE:

The favourable prognosis of certain histologic subtypes of invasive carcinomas is now well recognized. Patients with invasive duct carcinoma of breast can be divided into broad prognostic factors according to histologic type:

Excellent prognosis: Tubular, cribriform, mucinous and tubulolobular carcinoma

Good prognosis: Mixed- tubular, Mixed- ductal NOS and special type and classic lobular carcinoma

Average prognosis: Mixed lobular, medullary and atypical medullary carcinoma

Poor prognosis: ductal NOS,mixed ductal and lobular, solid lobular carcinoma and grade 3 basal type carcinoma

LYMPHOVASCULAR INVASION:

1. Tumor emboli in clear spaces with endothelial lining.
2. Tumor emboli do not assume the same shape as the lymphovascular vessel.

Lymphovascular invasion is specifically of prognostic value in node negative disease and predicts local recurrence after breast conservation surgery[64] and flap recurrence following mastectomy[65].

HISTOLOGIC GRADE:

The essential aspect of oncologic pathology has been the recognition that the morphological appearance of tumors can be correlated with the grade of malignancy[58].

In 1925 first analysis in breast carcinoma was carried out by Greenhough who classified into three grades based on eight histological factors. In 1928 Scarff and Patey reassessed and found that tubule formation, hyperchromatic figures and regularity in size and shape of nuclei were the three important factors .

In 1950 Bloom favoured greenhough method and used the three factors- degree of tubule formation,regularity in size and shape of nuclei

and mitotic figures. In 1957 Bloom and Richardson developed the grading system in the form of numerical scoring system.In 1968 Patey and Scarff method along with Bloom Richardson method has become the Bloom Richardson grading Method[58].

In 1982 Elston modified the Scarff Bloom Richardson method to improve the assessment of mitotic figures. In 1991 Nottingham improved the objectivity of histological grading for assessment of each component factor. Leslie et al states that histologic grading of invasive breast cancer is a valuable prognostic feature. It is a simple low cost method which serves to evaluate morphological characteristics semiquantitatively.

Jean.F.Simpson et al states that the Nottingham Combined Histologic Grade (NCHG) system is a modification of the Scarff-Bloom-Richardson grading system,which combines the analysis of glandular differentiation, nuclear grade, and mitotic activity[40]. The NCHG has been shown to be predictive and reproducible and is in widespread use[43,44].

John s.Meyer et al state that most useful components of grading system which assess the prognosis are mitotic index and tubularity. The Nottingham–Bloom–Richardson system can be improved by reducing the cutoff for mitotic index and by counting 20–30 fields rather than 10 high-power fields[45].

Pinder et al analyzed histologic grade as a prognostic factor and as a indicator of response to chemotherapy in an International Breast Cancer Study Group trial involving 146 pateints[46]. The National Cancer Institute consensus conferences recognized that the essential factors in the treatment decisions for node-negative breast carcinoma were assessment of tumor grade and proliferative activity[47].

Histologic grade was reported to be the significant prognostic factor in the response of stage 2 patients to adjuvant chemotherapy[48].It was also a indicator of treatment response among patients who receive endocrine treatment for systemic recurrence[49,5]

Histologic grading should be done in formalin fixed paraffin embedded tissue sections. Delayed formalin fixation will affect the assessment of mitosis. 6 hour delay in fixation reduce the viable mitosis by 76%.The ideal fixative is 10% neutral phosphate buffered formalin (pH-7).The sections should be cut at 4-5 microns thickness. Conventional staining with haematoxylin and eosin is sufficient [58]

Grading is carried out in invasive carcinoma and not in in-situ component. Elston and Ellis [51] advocated grading to all histological types of Invasive carcinoma regardless of morphologic type and this practice have now gained general acceptance. Pure tubular, Mucinous and cribriform carcinomas were graded as grade1.Medullary carcinoma

was placed under grade 3 .Invasive lobular carcinomas were also graded using this method. Pleomorphic variant of lobular carcinoma were graded as grade 3. Further, for some special types such as mucinous and tubular mixed carcinoma , grading provides a more appropriate evaluation of prognosis rather than the histologic type. For mixed carcinoma, grading proved to be a better prognostic factor than typing alone[70].The most widely accepted Nottingham modification of Scarff Bloom Richardson grading system was used.They include

- Tubule formation
- Nuclear pleomorphism
- Mitotic count

TUBULE FORMATION:

Tubular structures are those with central lumen surrounded by polarized tumor cells. Solid clusters with reversal of polarity seen in micropapillary carcinoma should be scored 3 for tubule formation. All areas of the tumor should be scanned and the proportion of the tumor occupied by definitive tubular structures with central luminal space is assessed. Care should be taken not to mistake clefts induced by shrinkage for glands/tubule.

NUCLEAR PLEOMORPHISM:

Assessment of nuclear pleomorphism is the most subjective element of histological grade. Fritz Rank et al states that criteria for nuclear pleomorphism includes irregularity in size, shape and staining of nuclei[52].

Nuclear pleomorphism is a morphological measurement of tumor differentiation at the cytological level and from the genetic point of view, it is a indirect measurement of aneuploidy and gentic instability.To establish the degree of objectivity, we use the size of normal epithelial cells in the adjacent breast tissue as a reference. When normal epithelial cells were not seen, stromal lymphoid cell is used as a reference for cell size and shape

MITOTIC COUNT:

They reflect the proliferative activity of the tumor and the most prognostically significant component of the grading system. Mitotic rate was reported to be the most important feature of Bloom Richardson grading system by Parham et al[53].Jannink compared four methods for measuring mitoic activity and concluded that the traditional mitotic activity index was preferable[53]. In a study of these individual parameters,

it has been concluded that the presence of mitotic activity is the strongest predictor of decreased survival. [40]

Van Deist et al offered a detailed specification for mitotic figures[54]

Morphological criteria for mitotic figures:

- Absent nuclear memberane
- Clear hairy extension of the nuclear material, either clotted(beginning metaphase) in a plane (metaphase/ anaphase) or in separate clots (Telophase)
- The surrounding cytoplasm should not be eosinophilic
- Two parallel clearly separate chromosome clots should be counted as seperate mitosis

Triangular or spiky nucleus with eosinophilic cytoplasm favours apoptosis. They indicate individual cell necrosis rather than proliferation The Nottingham modification excludes the hyperchromatic nuclei when this is the only mitosis related feature. Mitotic score depends on the number of mitosis per high power field. The size of the high power field should be standardized.

Variations in field size had the greatest effect on the scoring of mitotic counts when mitotic count per mm^2 was between 7 and 20.As a

method of overcoming this difficulty, a table has been created listing mitotic rates per 10 high per fields for different microscopes[71,72] and a conversion factors have developed for converting counts per 10 high power field to counts per mm[73] . Field areas ranges from 0.071mm^2 to 0.385mm^2 with an objective of 40 X. An important variable in these calculations is the field number,or the index, a factor that varies among microscopes and can be obtained from manufacturer.

Kupio and collan demonstrated substantial variation of grading in Bloom Richardson system depending on field size and mitoic rate[55.] A Minimum of 10 fields at the periphery of the tumor has to be counted. If there is variation in mitotic figures in different areas of the tumor, the least differentiated area should be taken into account. The preliminary assessment of the section should be carried out under low power to assess the least differentiated area. Start counting when one notices atleast one mitotic figure and then proceed to 10 consecutive non-overlapping fields

Robinson et al reported that grade 2 tumors comprise about 60 % of invasive breast Cancer[56].Chabra et al reported that maximum number of cases as grade 2(52%) followed by grade 1(30%) and grade 3(18%)[57].Assesment of tubular differentiation is done on the overall apearence of the tumor whereas nuclear appearance including mitotic figure are evaluated on the least differentiated part of the tumor

OTHER PROGNOSTIC FACTORS:

NECROSIS:

Tumor necrosis is defined as the presence of confluent necrosis of any dimension that could be distinguished at intermediate grade. Vischer et al support the view that widespread necrosis is a unfavourable feature in invasive duct carcinoma of breast

APOPTOSIS:

Jonnsur et al observed bcl-2 observation expression in low grade tumors. Silverstrini et al [76]shows that bcl-2 staining is in inversely related to proliferative activity. Most investigators reported that bcl-2 immunoreactivity was in low grade tumors[74,75].Zhang et al concluded that bcl-2 expression is a better response to hormone therapy and a favourable prognostic factor regardless of nodal status.

EXTENT OF INTRAUCTAL CARCINOMA:

Silverberg and Chitale observed a trend of decreased nodal metastasis and favourable prognosis when intraductal carcinoma is extensive[77].Recurrence occurs more often in breast after lumpectomy and

radiation therapy in women who have comedo intraducatal carcinoma or when there is extensive intraductal carcinoma within and around an invasive tumor that comprises at least 25% of neoplasm.

IMPACT OF NEOADJUVANT THERAPY ON PROGOSIS:

A Study of 372 pateints with locally advanced breast carcinoma found that 60 Patients had complete pathologic response after neoadjuvant therapy [59]. Factors predictive of complete pathologic response were poorly differentiated nuclear grade, negative estrogen receptors, poorly differentiated histologic grade. The fundamental observation in treatment effect is a decrease in tumor cellularity. Rajan et al observed a decrease in tumor cellularity from 40% in pretreatment biopsies to 10% in tumor resected after chemotherapy[60].In most extreme situation, no residual carcinoma may be detectable, an occurrence reported in 6.7%[61] and 10% [62].

Healed sites of previously infiltrating carcinoma characterized by fibrosis, stromal edema, increased vascularity and chronic inflammatory cell infiltrate. Stromal elastosis may be prominent after treatment. Foci of intraductal carcinoma and lymphatic tumor emboli may be relatively resistant to treatment. This was supported by study by sharkey etal[63].cytological alterations includes enlarged cells with increased cytoplasmic volume. The cytoplasm contains cytoplasmic vacuoles and

eosinophilic granules.cell borders are well defined with shrinkage of cell from the stroma. Multinucleated giant cells and abnormal mitotic figures were seen.

An analysis comparing the histologic grading in pre and post chemotherapy specimens did not reveal significant differences[82] whereas some others reveal upgrading of tumor in 32% of cases[63].Further it was concluded that grading of treated carcinomas may not be predictive of prognosis after therapy.

MATERIALS AND METHODS

This prospective study was carried out in 50 cases of invasive duct carcinoma of breast for a period of one year from August 2011-july 2012.This study was done in department of pathology, Coimbatore medical college Hospital, Coimbatore. In all these cases the cytological diagnosis were confirmed by histological examination.

INCLUSION CRITERIA:

1. Female patients with palpable breast mass.
2. Patients who were proved to have ductal carcinoma of breast by FNAC.
3. Patients whose cytological diagnosis of Invasive duct carcinoma was confirmed in histological specimens
4. Other special types of ductal carcinoma were also included.

EXCLUSION CRITERIA :

1. Known cases of invasive duct carcinoma of breast who have received chemotherapy or radiotherapy

2. Patients those who do not have histological confirmation

3. Male patients with ductal carcinoma of breast.

4. Patients who underwent lumpectomy alone.

DATA COLLECTION :

Female patients with palpable breast lesions were selected. Patients' age, tumor location, tumor size as examined clinically were recorded. The patients were classified into three categories based on age

1) Pre-menopausal(<47)

2) Menopausal (47-52)

3) Post-menopausal(>52).

The consent was obtained from the patient.

FNA PROCEDURE:

The FNA Procedure was done by the Faculty and Postgraduates of Department of pathology, Coimbatore medical college using 23 gauge needle with or without 10 ml disposable syringe by non-aspiration technique. The aspirated material was expressed on slide, smeared and

fixed with 95% alcohol for 20 minutes. These wet fixed slides were stained with Papanicolaou method and Haematoxylin and eosin.

The procedures of Papanicolaou and Haematoxylin and eosin stain were as follows:

Papanicolaou stain:

Fix in 95% Isopropyl alcohol-20 minutes

80% Isopropylalcohol-1minute

70% Isopropylalcohol -1 minute

50% Isopropylalcohol -1 minute

Rinse in water-10 minutes

Harris haematoxylin-7 minutes

Rinse in tap water-10 minutes

Differentiate in 1% acid alcohol

Blueing in tap water-10 minutes

70% Isopropylalcohol - 5 minute

90% Isopropylalcohol - 5 minute

OG-6 - 2 minutes

95% Isopropylalcohol -1 minute

95% Isopropylalcohol -1minute

95% Isopropylalcohol -1 minute

EA-50 - 4 minutes

95% Isopropylalcohol -1 minute

95% Isopropylalcohol-1 minute

95% Isopropylalcohol -1 minute

Xylene:Alcohol(1:1)-5 minutes

Xylene- 10 minutes

Mount with DPX

The Procedure for Haematology and eosin stain in cytology:

Fix in 100% isopropyl alcohol -20minutes

Haematoxylin-15 minutes

Rinse in tap water-10 minutes

Eosin-7 dips

Rinse in tap water

Dry, Xylene and mount

The stained smears were evaluated and diagnosed as ductal carcinoma of breast when there is definite features of malignancy like cellular dissociation, nuclear pleomorphism, nuclear abnormalities, absence of bare nuclei. Any special variants of ductal carcinoma were also included. These slides were graded based on grading system proposed by Robinson. Those smears with suspicious cell clumps and features suggestive of ductal hyperplasia were excluded.

Robinson cytological grading system:

	SCORE 1	**SCORE 2**	**SCORE 3**
Cell Dissociation	Mostly in clusters	Single cells and clusters	Mostly in single cells
Nuclear size	1-2 times the size of RBC	3-4 times the sizeof RBC	>5 times the size of RBC
Cell uniformity	Monomorphic	Mildly pleomorphic	Pleomorphic
Nucleoli	Indistinct/small	Noticeable	Abnormal
Nuclear margin	Smooth	Slightly irregular/folds/grooves	Buds and clefts
Chromatin pattern	Vesicular	Granular	Clumping and clearing

The value between 1 and 3 is given for every factor analyzed. The sum of these values gives the total score. Total score ranges between 6 and 18. According to which grading was done.

Grade 1: Score 6- 11

Grade 2: Score 12-14

Grade 3: Score 15-18

Modified radical mastectomy specimens including axillary clearance fixed in 10% formalin were obtained. The tumor size and the tumor location were assessed in the gross specimens.

Paraffin embedded sections obtained by routine procedures were cut at thickness of 3 microns using Leica microtome .These slides were routinely stained with haematoxylin and eosin stain.

The slides were evaluated and the diagnosis of invasive duct carcinoma was confirmed. Special types of ductal carcinoma other than classical invasive duct carcinoma NOS were also included in this study Histological grading was done using Nottingham modification of Scarff Bloom Richardson method. This system considers three parameters .They include tubule formation, nuclear pleomorphism and mitotic rate.

Modified Bloom Richardson Histologic grading

	1	2	3
Tubule formation	>75%	10-75%	<10%
Nuclear pleomorphism	Small regular uniform cells	Moderate increase in size and variability	Marked variation
Mitotic count	<9	10-19	>20

Each parameter was assigned a score of 1 -3.Each score was added to give a total score of 3- 9. In the present study mitotic count was done in 0.59mm field diameter Olympus microscope 24mm eyepiece with a 40x objective gives a field diameter of 24 ÷ 40 = 0.59mm .Number of mitosis were counted per 10 high power field. Mitotic counts .according to the field diameter were given in the appendix 2.According to the score they are graded.

Grade 1: Score 3-5

Grade 2: Score 6-7

Grade 3: Score 8-9

The axillary lymph nodes received were also processed in a similar manner and assessed for metastasis. Statistical analysis was done to examine the degree of association between the cytologic and histologic grading system using Chi square test. Also the tumor grades were correlated with other prognostic factors which includes age of the patient, tumor size and lymph node status independently. The P value < 0.05 was considered to be statistically significant.

OBSERVATIONS AND RESULTS

Total of 50 cases were studied and the following observations were obtained.

AGE DISTRIBUTION:

The patients age ranges from 32-86 years with a mean age of 52.52 years. The minimum age and the maximum age reported in the present study was 32 and 86 years respectively.

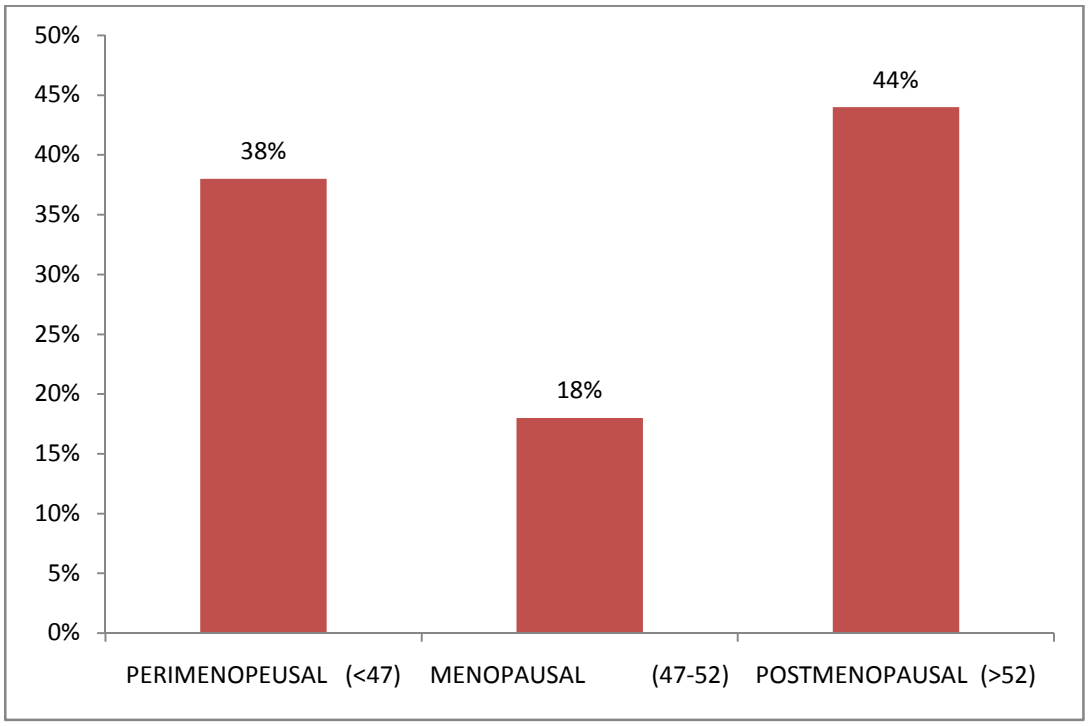
The patients age group were divided into three age groups

Table 1:Distribution of cases according to different age groups

AGE GROUP	NUMBER(%)
PREMENOPEUSAL (<47)	19(38%)
MENOPAUSAL (47-52)	9(18%)
POSTMENOPAUSAL (>52)	22(44%)

According to this table, majority of cases belong to the post menopausal age group (44%), 38% of case in premenopausal age group and the 18% of cases in the menopausal age group.

Chart – 1: Distribution of cases according to different age groups



TUMOR SIZE:

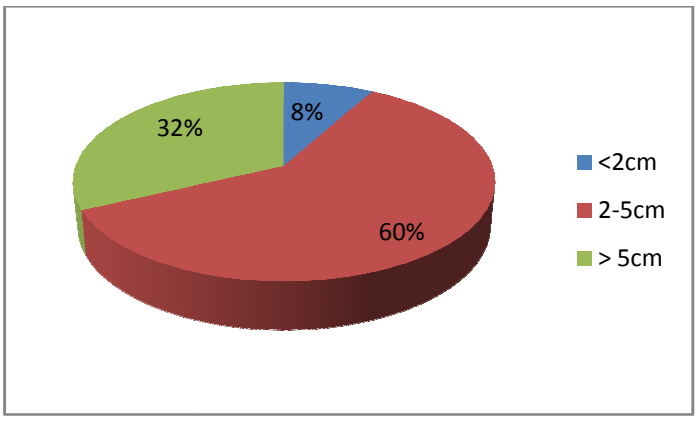
The cases were divided into three categories based on tumor size according to TNM staging

Table 2: Distribution of cases according to tumor size

TUMOR SIZE(cm)	NUMBER
<2	4 (8%)
2-5	30 (60%)
>5	16 (32%)

Of these fifty patients,four (8%) of them have tumor size <2cm.thirty(60%) patients have tumor size between 2 and 5 cm. sixteen(32%) of them have tumor size >5cm.

Chart – 2: Distribution of cases according to tumor size



LYMPHNODE STATUS:

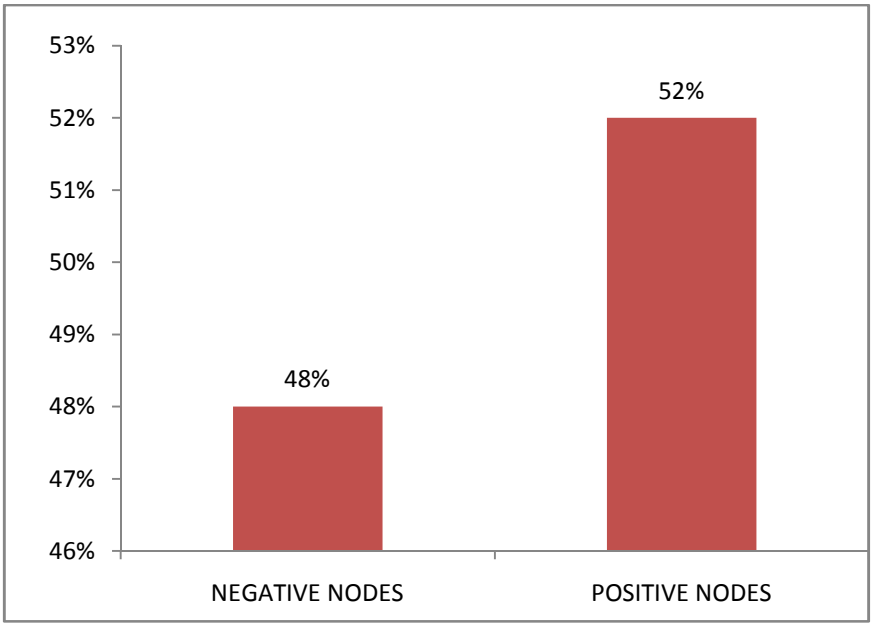
The lymph node status was classified into positive and negative nodes

Table 3: Distribution of cases according to lymph node status

NODAL STATUS	NUMBER OF CASES(%)
NEGATIVE NODES	24(48%)
POSITIVE NODES	26(52%)

Of the fifty cases, twenty four(48%) cases were node negative Among the twenty six node positive cases, ten cases had 1-3nodes, fourteen cases had 4-9 nodes, two cases had more than 10 nodes.

Chart-3: Distribution of cases according to lymph node status



TUMOR LOCATION:

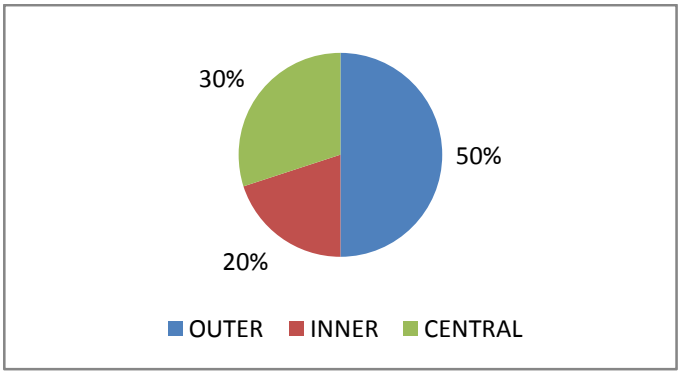
According to the location of the tumor, they have been classified into outer, inner and central quadrant

Table 4:Distribution of cases according to the tumor location

TUMOR LOCATION	NUMBER OF CASES
OUTER	25 (50%)
INNER	10 (20%)
CENTRAL	I5 (30%)

In this study of fifty cases, majority of the cases were located in the outer quadrant (50%) followed by central quadrant tumors (30%) .20% of cases were located in the inner quadrant

Chart – 4: Distribution of cases according to the tumor location



CYTOLOGICAL GRADE:

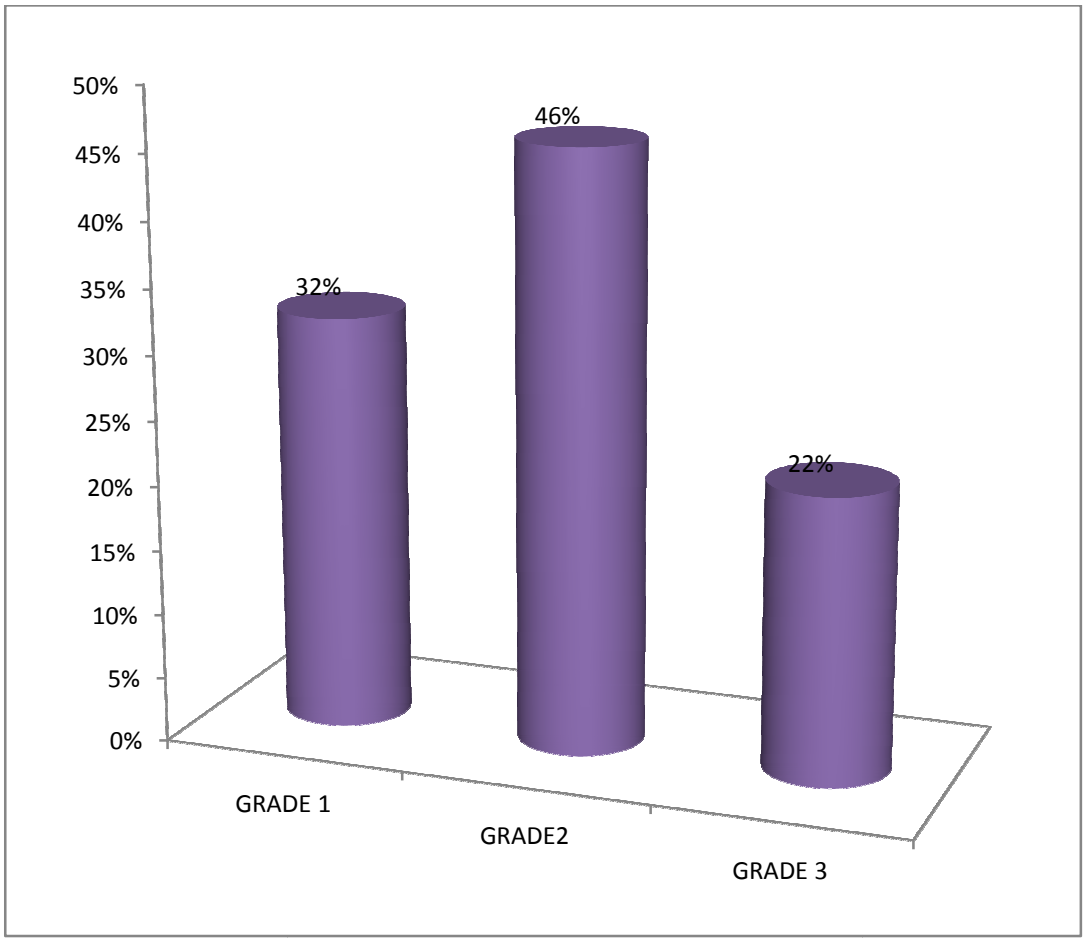
According to Robinson grading system, the cytological samples were graded into three groups

Table 5: Distribution of cases with regard to cytological grade

CYTOLOGIC GRADE	NUMBER
GRADE 1	16(32%)
GRADE 2	23(46%)
GRADE 3	11(22%)

Of the fifty cases, majority of the cases belong to grade 2(46%) with 23 cases, next was grade 1(32%) with 16 cases followed by grade 3 (22%) with 11 cases. Few special types were also reported .This includes mucinous carcinoma which was graded as grade 1,papillary carcinoma under grade 2.one of the grade 1 carcinoma show a uniform dispersed cells with plasmacytoid morphology. One of the ductal carcinoma with grade 2 nuclear features show plenty of lymphocytes in the background.

Chart -5: Distribution of cases with regard to cytological grade



HISTOLOGICAL GRADE:

According to Nottingham modification of Scarff Bloom Richardson grading, fifty cases were categorized under three categories

Table 6:Distribution of cases with regard to histological grade

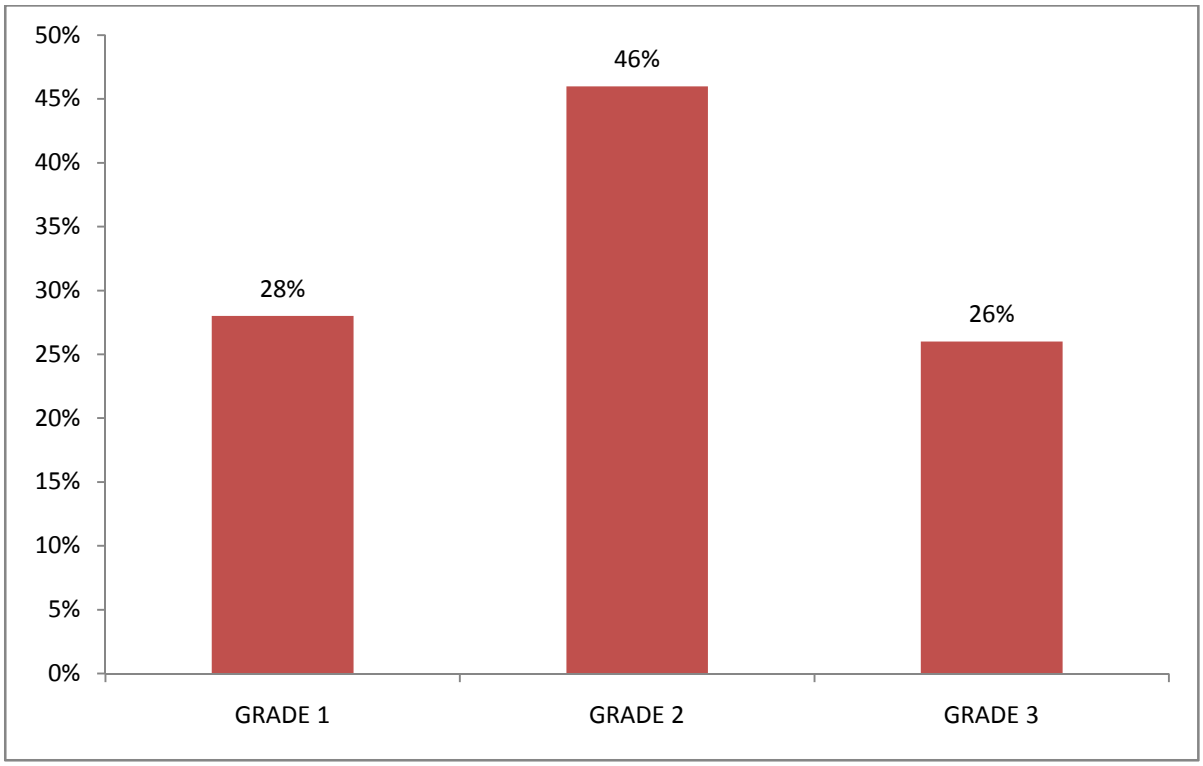
HISTOLOGIC GRADE	NUMBER(%)
GRADE 1	14(28%)
GRADE 2	23(46%)
GRADE 3	13(26%)

In this study of fifty cases, majority of tumors were grade 2 tumors with twenty three cases (46%),fourteen cases were under grade 1(28%) and thirteen cases were classified as grade 3(26%).

Among these fifty cases, there were three special types of invasive duct carcinoma and the rest of the forty seven cases were Invasive duct carcinoma,NOS. The special types include mucinous carcinoma under grade 1, papillary carcinoma under grade 2, One of the grade 2 invasive ductal carcinoma NOS show neuroendocrine differentiation. One of the grade 3 ductal carcinoma diagnosed in cytologic samples as ductal

carcinoma with plenty of lymphocytes in the background was diagnosed to be atypical medullary carcinoma.

Chart-6: Distribution of cases with regard to histological grade



CORRELATION OF CYTOLOGIC GRADE WITH HISTOLOGIC GRADE:

The cytological grade of the tumor was correlated to the histologic grade to assess the concordance between the two grading systems.

Table 7:Correlation of cytologic grade with histologic grade

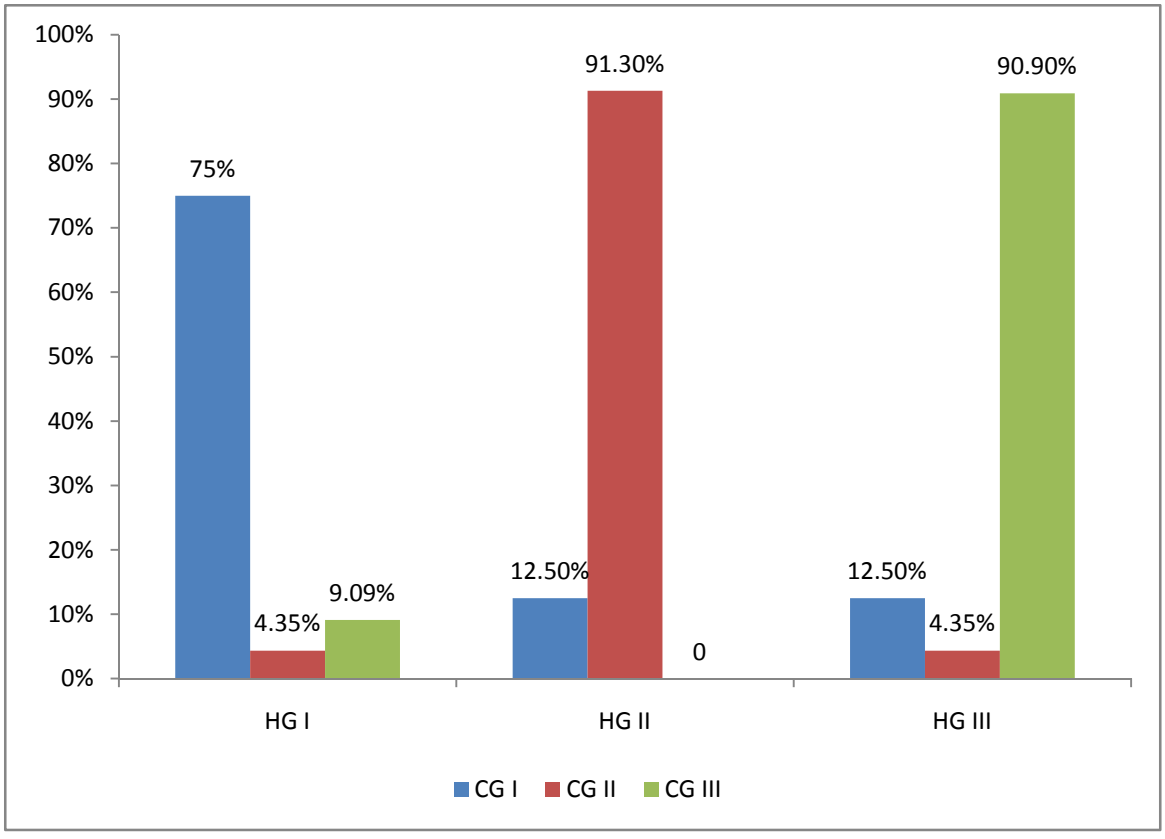
		HISTOLOGIC GRADE			**TOTAL**
		1	2	3	
CYTOLOGIC GRADE	1	12(75%)	(12.5%)	(12.5%)	**16**
	2	1(4.35%)	21(91.3%)	1(4.35%)	**23**
	3	1(9.09%)	0	10(90.9%)	**11**
		14	23	13	**50**

CHI-SQUARE TOTAL = 60.04 DF = 4 Significant at 1% level (P<0.01)

In this study of fifty cases, forty three cases(86%) show concordance between cytologic and histologic grading systems . Rest of the seven cases (14%) show discrepancy. Among the 16 grade 1 tumors,

twelve cases (75%) show concordance with the histological grade whereas four cases were discordant. All these four cases were upgraded. Among the twenty three grade 2 tumors, two cases did not correlate, one was upgraded to grade 3 and the other was downgraded to grade 1.Rest of the twenty one grade 2 tumors show good concordance (91.3%).Grade 3 tumors show good concordance with ten cases (90.9%).Only one of the grade 3 tumor was downgraded to grade 1.In this study association between cytological grade and histological grade was statistically significant($p<0.01$).

Chart-7 Correlation of cytologic grade with histologic grade



CORRELATION OF THE CYTOLOGICAL GRADE WITH THE AGE OF THE PATEINT :

The cytological grade was compared with the age of the patient

Table 8:Correlation of cytological grade with the age of the patient

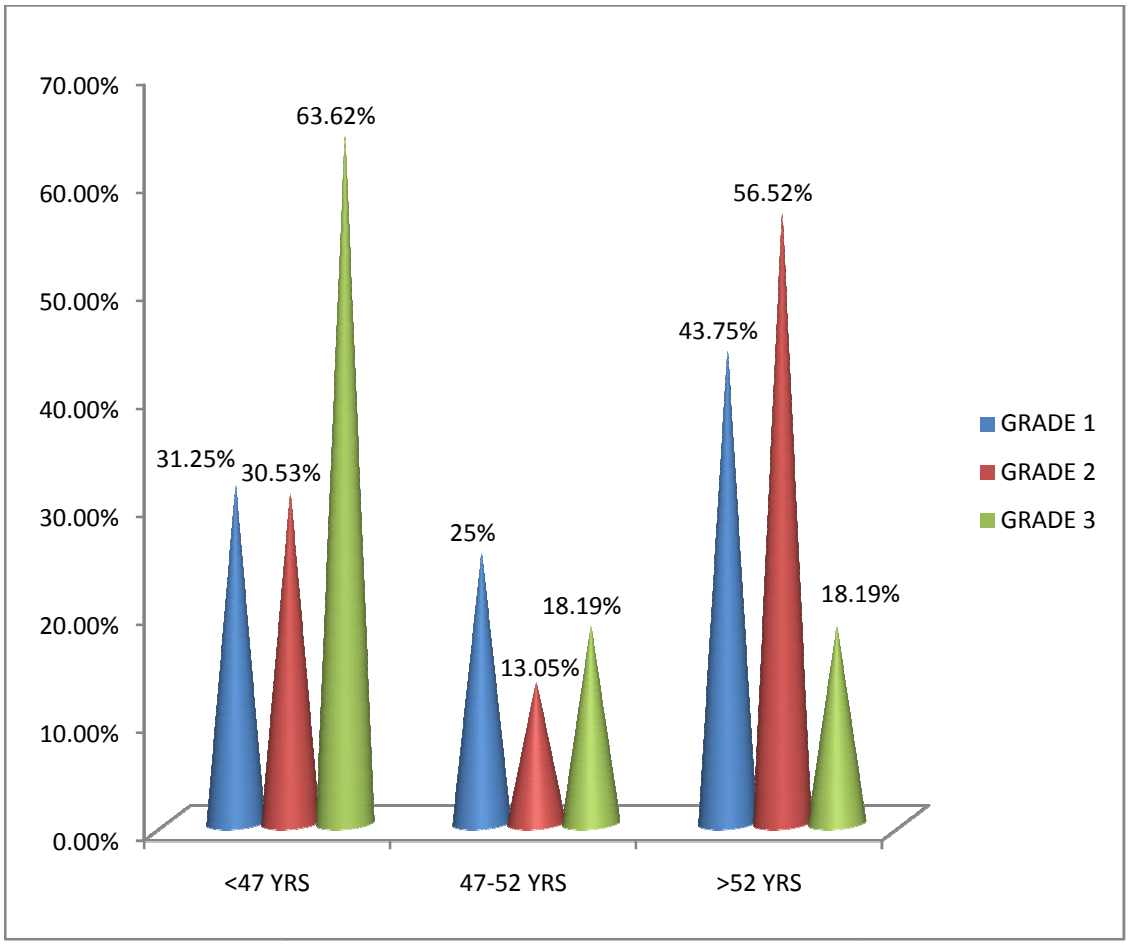
CYTOLOGICAL GRADE	AGE OF THE PATEINT			TOTAL
	<47 YRS	47-52 YRS	>52 YRS	
GRADE 1	5(31.25%)	4(25%)	7(43.75%)	16
GRADE 2	7(30.53%)	3(13.05%)	13(56.52%)	23
GRADE 3	7(63.62%)	2(18.19%)	2(18.19%)	11
	19	9	22	50

CHI-SQUARE TOTAL = 5.68 DF = 4 Not Significant (P>0.05)

The above table represents the correlation of cytologic grade with the age of the patient. It shows that grade 3tumors was seen to occur in younger age group (63.62%) which indicates aggressiveness of tumor in young age group. The minimum age reported was 32 years who shows grade 3 in both cytology samples and histology specimen .The cytologic

grade did not show a significant association with age of the patient($p>0.05$).

Chart-8: Correlation of cytological grade with the age of the patient:



CORRELATION OF CYTOLOGIC GRADE WITH TUMOR SIZE:

The cytologic grade was correlated with the tumor size to see whether high grade tumors were associated with large size tumors

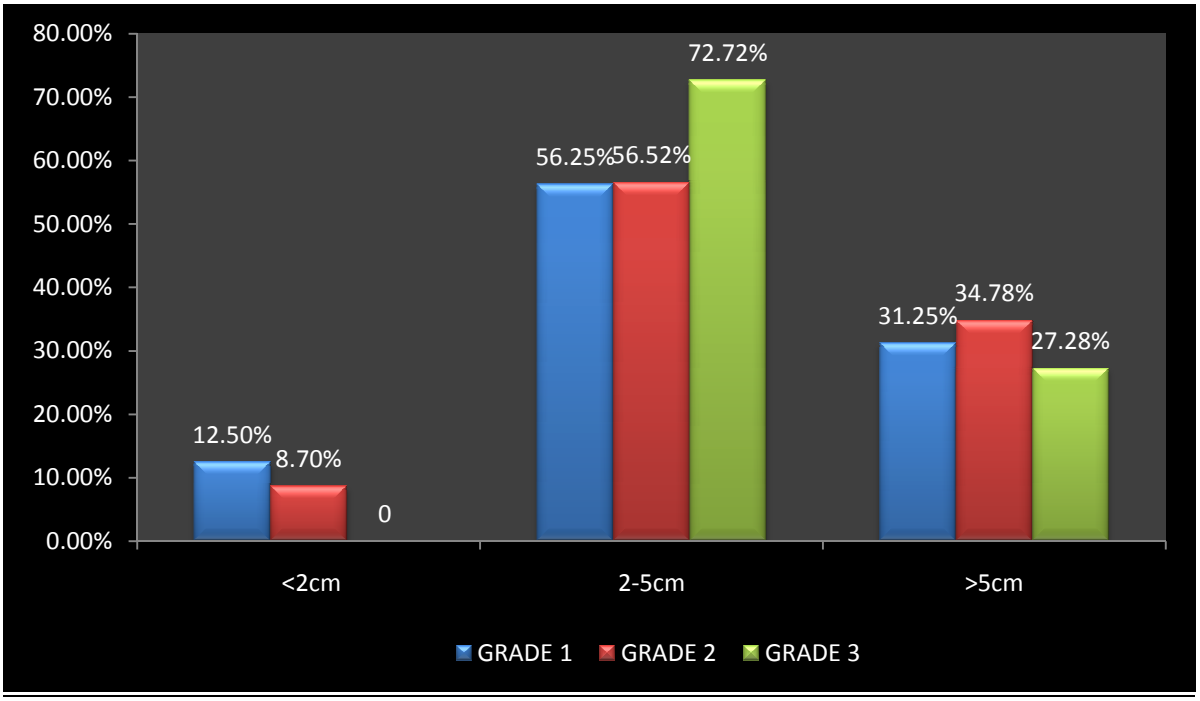
Table 9:Association between cytological grade and tumor size

CYTOLOGIC GRADE	TUMOR SIZE			TOTAL
	<2cm	2-5cm	>5cm	
GRADE 1	2(12.5%)	9(56.25%)	5(31.25%)	16
GRADE 2	2(8.7%)	13(56.52%)	8(34.78%)	23
GRADE 3	0	8(72.72%)	3(27.28%)	11
	4	30	16	

CHI-SQUARE TOTAL = 1.82 DF = 4 Not Significant (P>0.05)

Most of the tumors have a tumor size 2-5cm with 56.25% in grade 1,56.52% in grade 2 and 72.72% of grade 3 tumors respectively. The grade did not show a significant association with the tumor size (p>0.05)

Chart-9: Association between cytological grade and tumor size



CORRELATION OF CYTOLOGIC GRADE WITH LYMPHNODE STATUS:

The cytologic grade was correlated with the lymph node status to see whether high grade tumors were associated with increased lymph node positivity.

Table 10:Correlation of cytologic grade with lymph node status

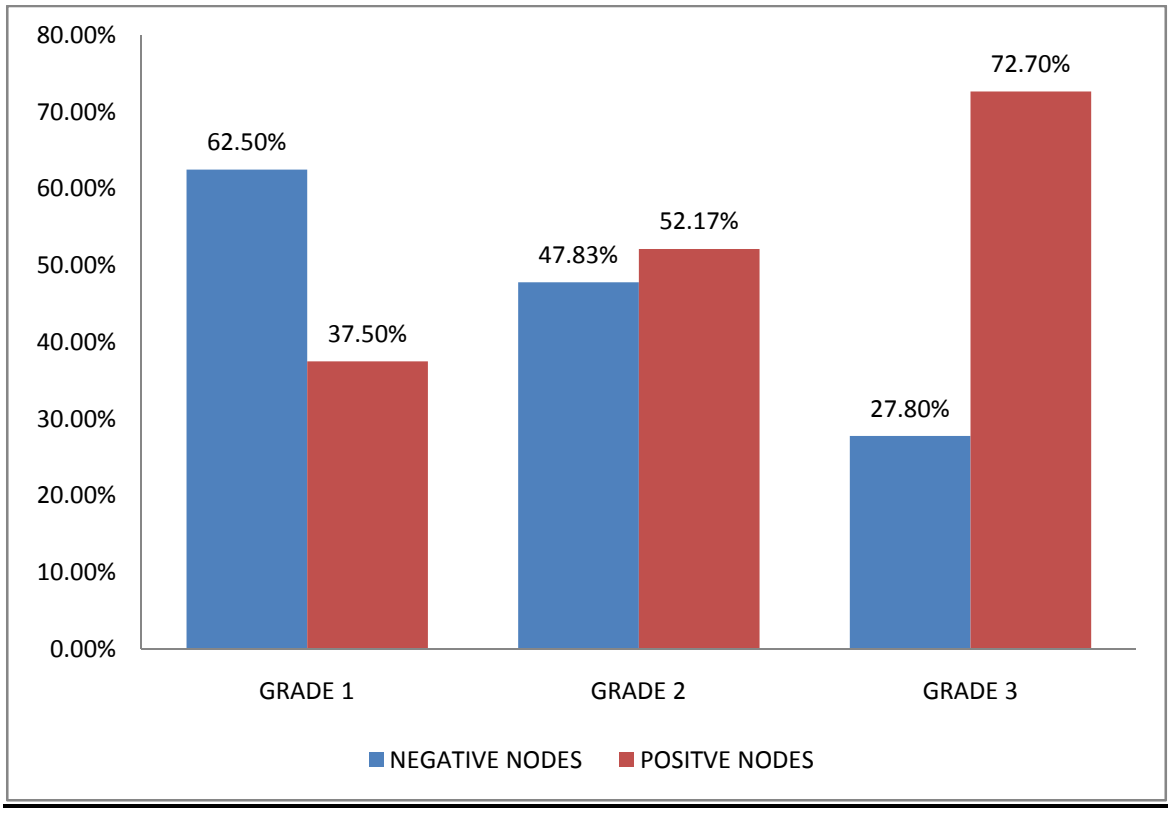
CYTOLOGIC GRADE	LYMPH NODE STATUS		TOTAL
	NEGATIVE NODES	POSITVE NODES	
GRADE 1	10(62.5%)	6(37.5%)	16
GRADE 2	11(47.83%)	12(52.17%)	23
GRADE 3	3(27.8%)	8(72.7%)	11
TOTAL	24	26	50

CHI-SQUARE TOTAL = 3.24 DF = 2 Not significant (P>0.05)

All the lymph nodes mentioned here were axillary lymph nodes. No cervical and internal mammary nodes were obtained.Lymph node metastasis was observed in 6 of grade 1, 12 of grade 2 and 8 of grade 3

tumors. Among the 16 cases of grade 1tumors 62.5% were node negative and only 37.5% were node positive. In contrast 72.7% and 52.17% of grade 3 and grade 2 were node positive respectively. Most of the tumors with more than four positive lymphnodes were grade 3 tumors. None of the grade 1 tumors had metastasis in more than 10 nodes. In the present study the association between cytological grade and lymph node status was not significant($p>0.05$).

Chart-10 Correlation of cytologic grade with lymph node status



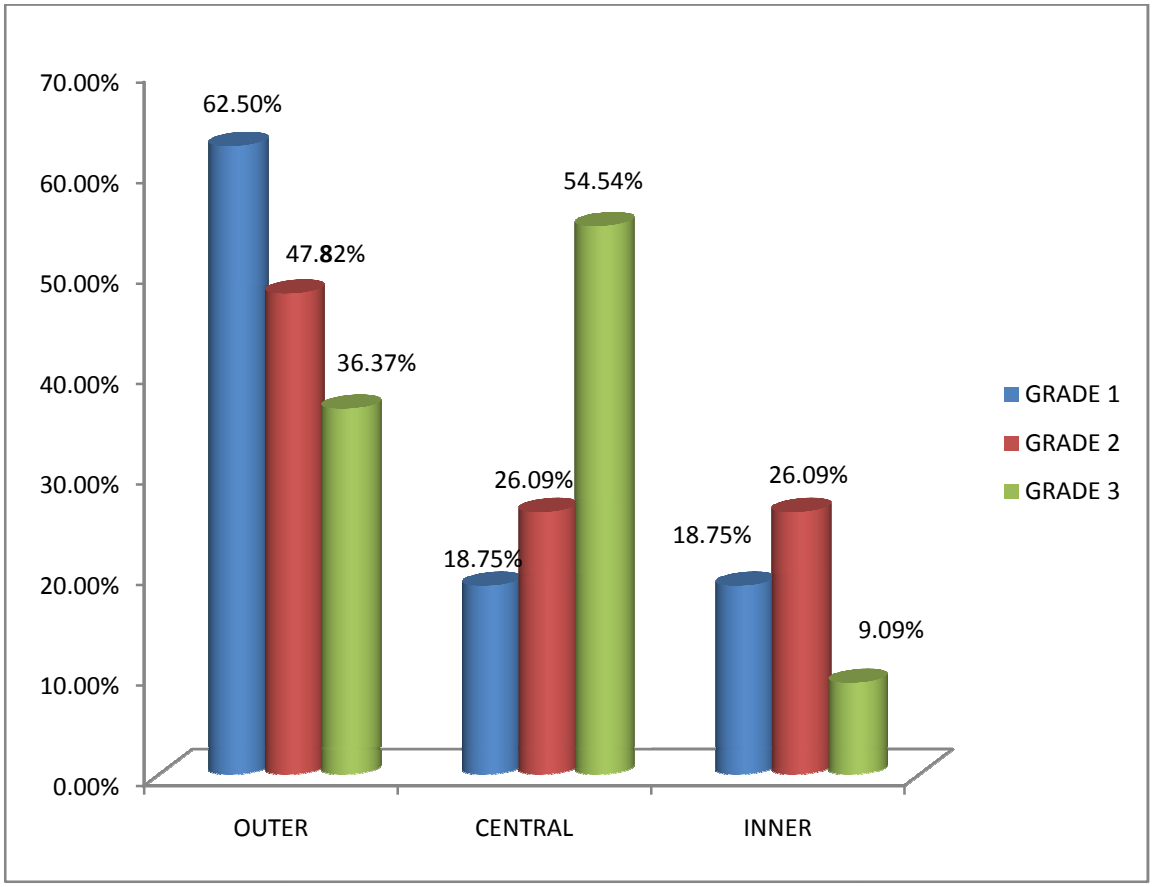
CORRELATION OF CYTOLOGICAL GRADE WITH THE TUMOR LOCATION

Table 11:Correlation of cytologic grade with the tumor location

CYTOLOGIC GRADE	TUMOR LOCATION			TOTAL
	OUTER	CENTRAL	INNER	
GRADE 1	10(62.5%)	3(18.75%)	3(18.75%)	16
GRADE 2	11(47.82%)	6(26.09%)	6(26.09%)	23
GRADE 3	4(36.37%)	6(54.54%)	1(9.09%)	11
	25	15	10	

Out of the fifty cases, most of grade 1 and grade 2 tumors were in outer quadrant with 62.5% and 47.82% respectively whereas most of grade 3 tumors were I the central quadrant(54.54%).

Chart-11: Correlation of cytologic grade with the tumor location



CORRELATION OF LYMPH NODE STATUS WITH TUMOR SIZE :

The lymph node status was correlated with the tumor size to see whether increase in tumor size was associated with increase in lymph node positivity.

Table 12:Association between lymph node status and tumor size

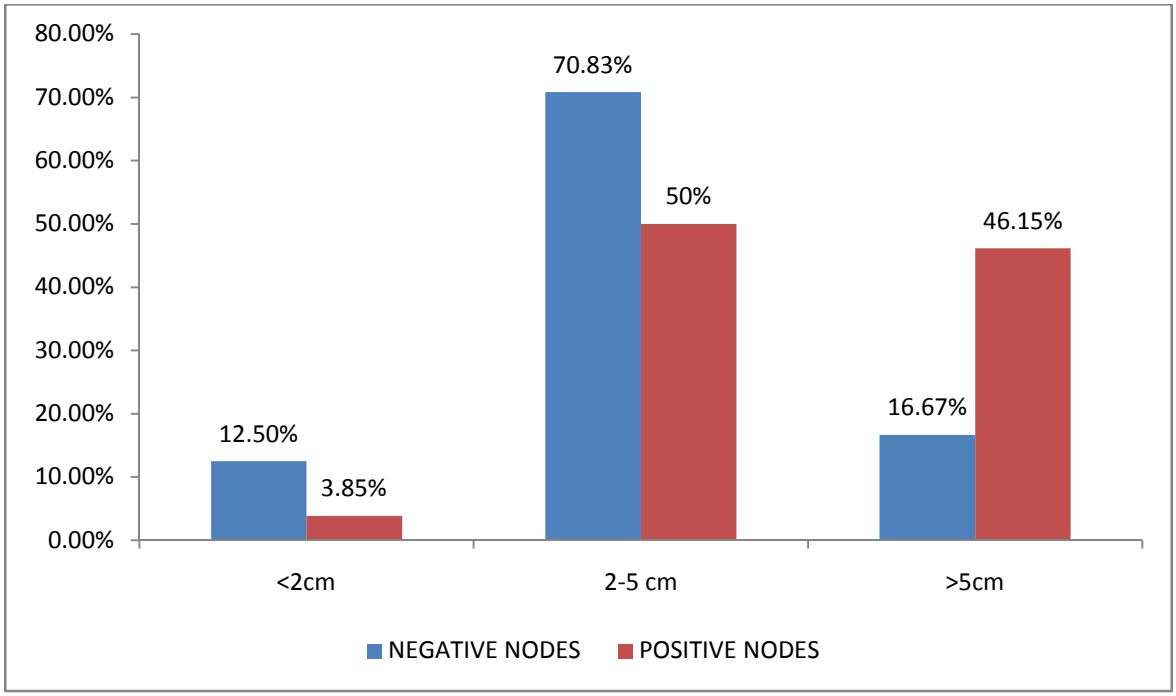
LYMPHNODE STATUS	TUMOR SIZE			TOTAL
	<2cm	2-5 cm	>5cm	
NEGATIVE NODES	3(12.5%)	17(70.83%)	4(16.67%)	24
POSITIVE NODES	1(3.85%)	13(50%)	12(46.15%)	26
TOTAL	4	30	16	

CHI-SQUARE TOTAL = 6.62 DF = 6 Not Significant (P>0.05)

This Table indicates that most of the tumors with positive lymph nodes have a size between 2-5 cm .Maximum numberof positive nodes was reported to be 18 and 15 and corresponding tumor size were 8.5and 7

cm respectively. In Mucinous carcinoma ,though the size was >10 cm all the twelve nodes were negative indicating a favourable prognosis .There was no significant association between the tumor size and lymphnode status(p>o.05).

Chart12: Association between lymph node status and tumor size



CORRELATON OF TUMOR LOCATION WITH LYMPH NODE STATUS:

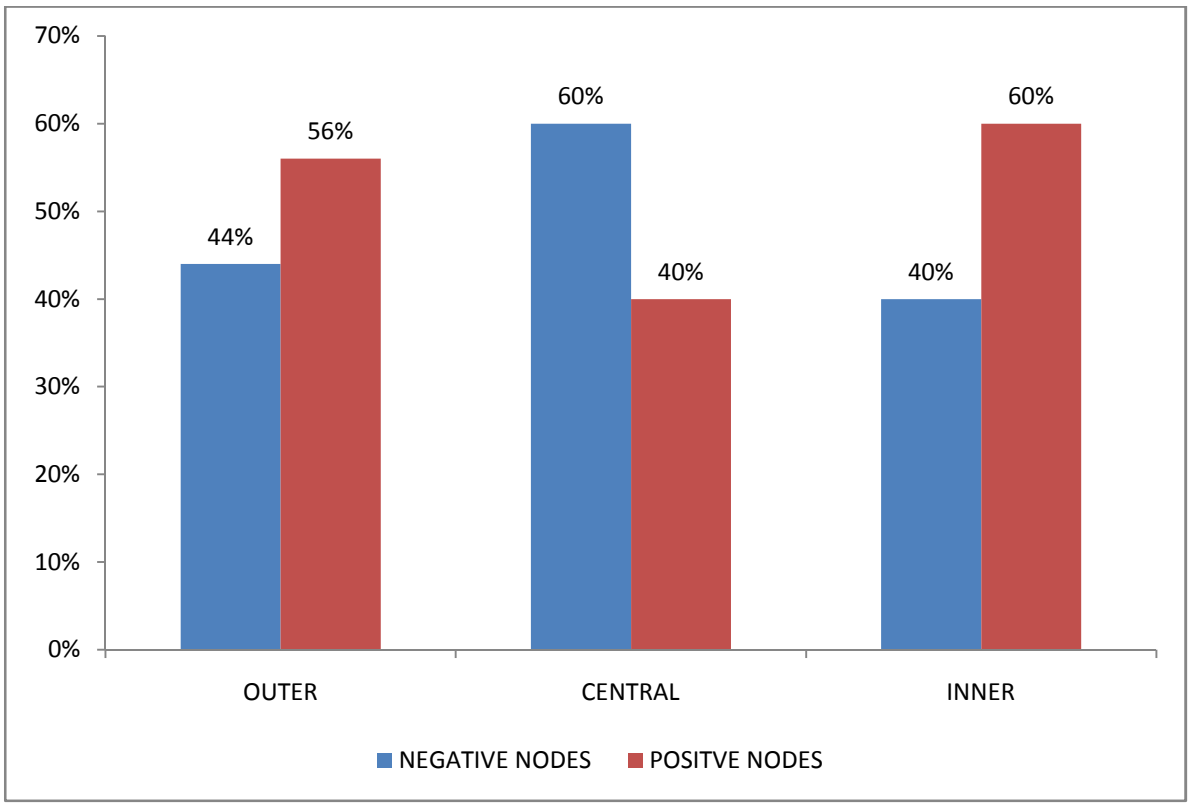
The lymph node status of the tumor was correlated with the tumor location to see whether the tumor in a particular location has more predilection for lymph node positivity

Table 13:Correlation of tumor location with lymph node status

TUMOR LOCATION	LYMPH NODE STATUS		TOTAL
	NEGATIVE NODES	POSITVE NODES	
OUTER	11(44%)	14(56%)	25
CENTRAL	9(60%)	6(40%)	15
INNER	4(40%)	6(60%)	10
TOTAL	24	26	50

This table indicates that 56% of outer quadrant tumors, 40% of central quadrant tumors and 60% of inner quadrant tumors were node positive.

Chart-13: Correlation of tumor location with lymph node status



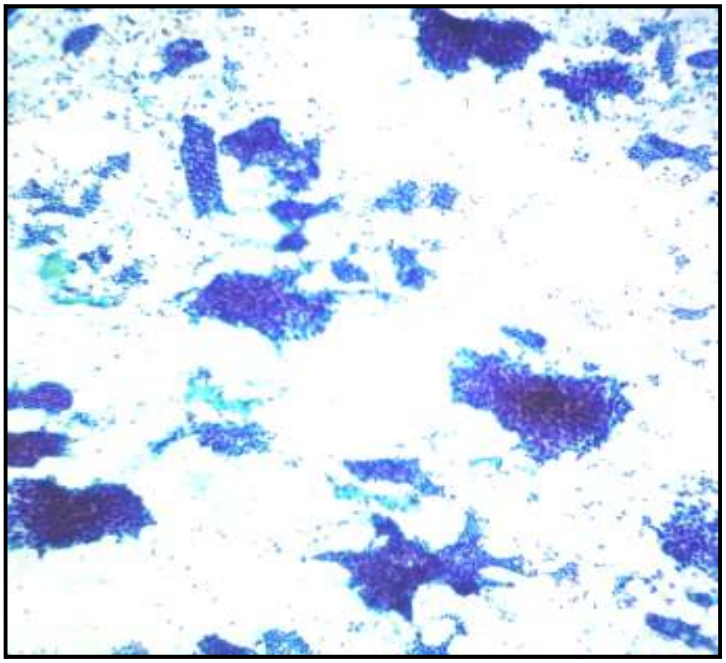


Fig 1: Malignant epithelial cells arranged in clusters (FNAC- Pap stain)10X

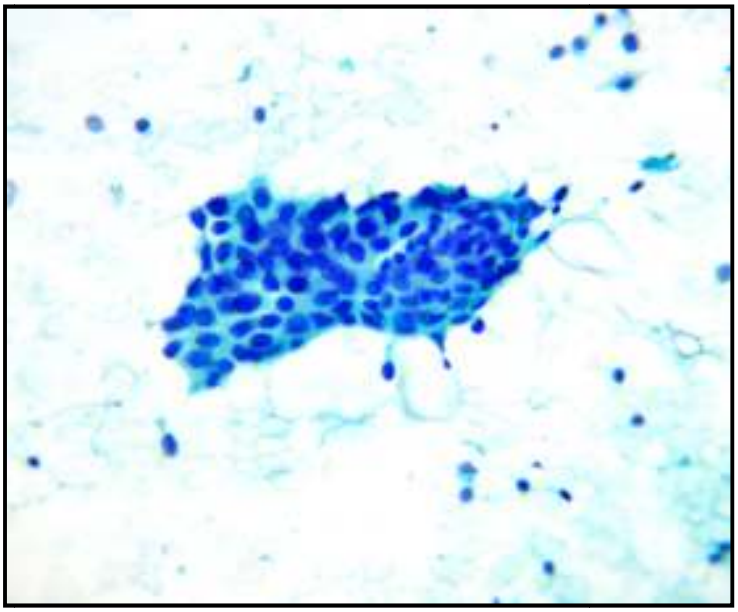


Fig 2:Monomorphic epithelial cells with smooth nuclear margins and inconspicuous nucleoli (FNAC-Pap stain)10X

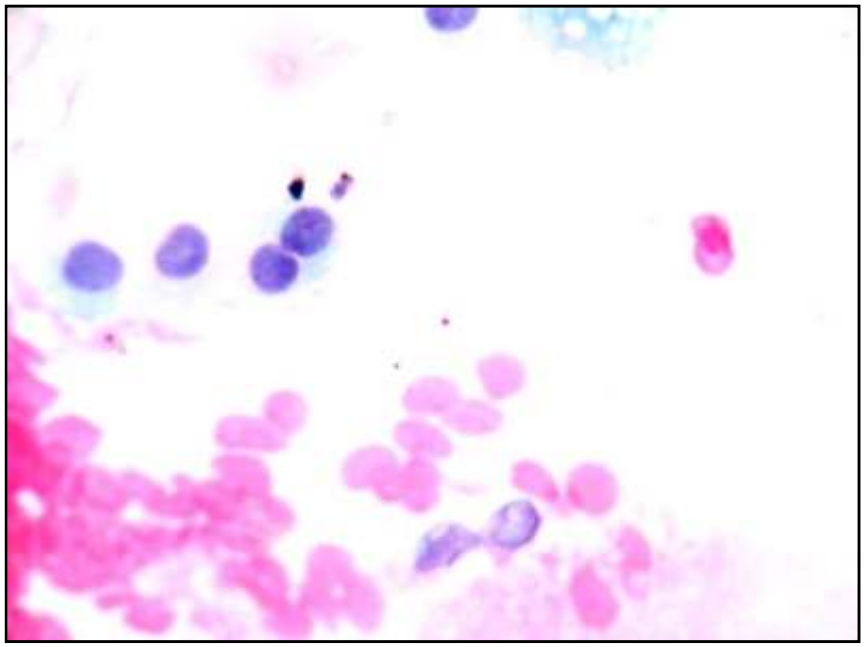


Fig 3: Malignant cell is 1-2 times the size of RBC (FNAC-H&E) 40 X

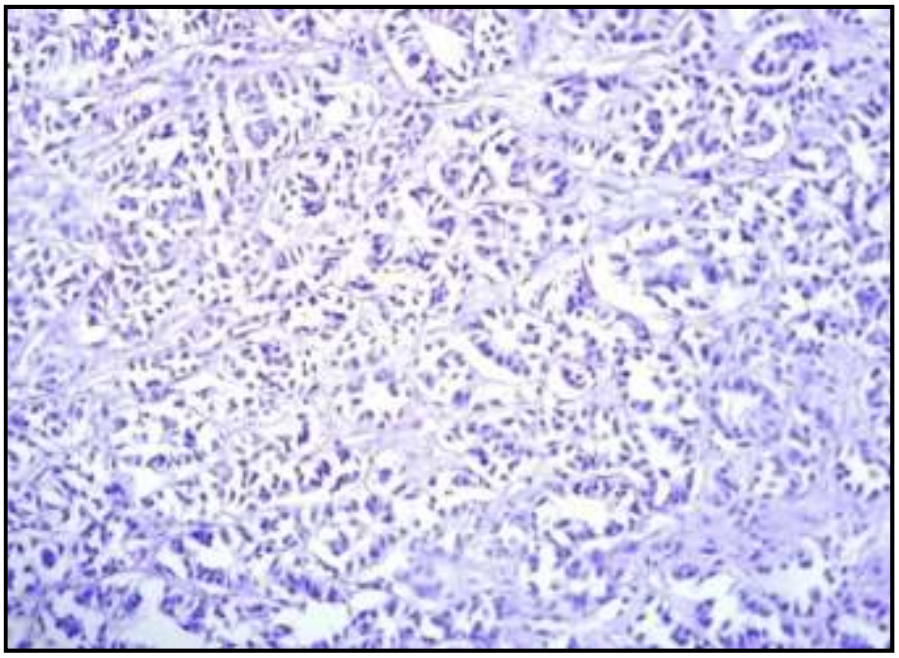


Fig 4: Tumor show >75% tubule formation (HPE-H&E) 10X.

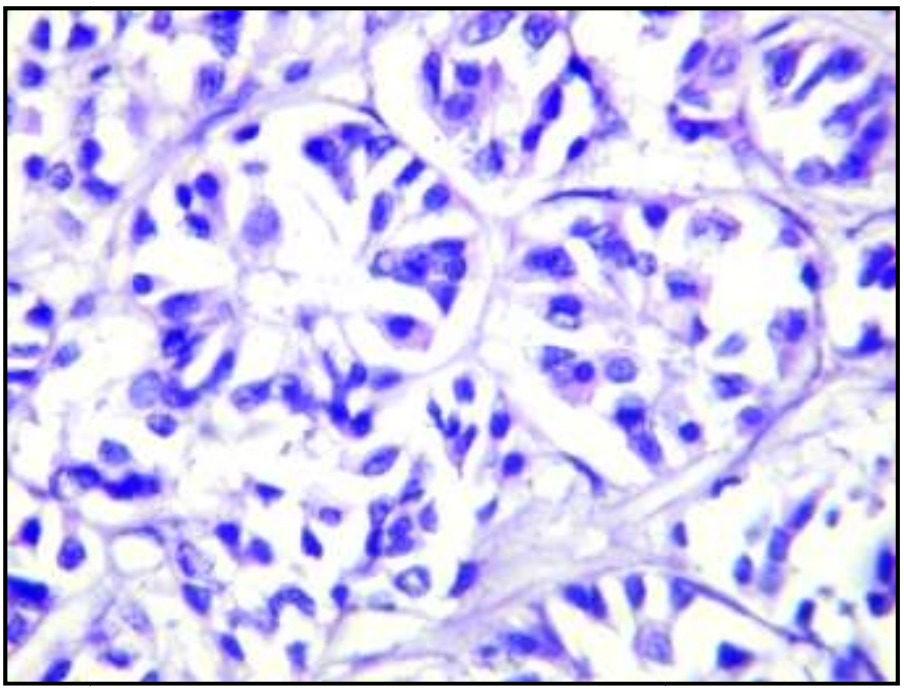


Fig 5: Small nuclei showing mild pleomorphism with regular nuclear membrane (HPE- H&E) 40X.

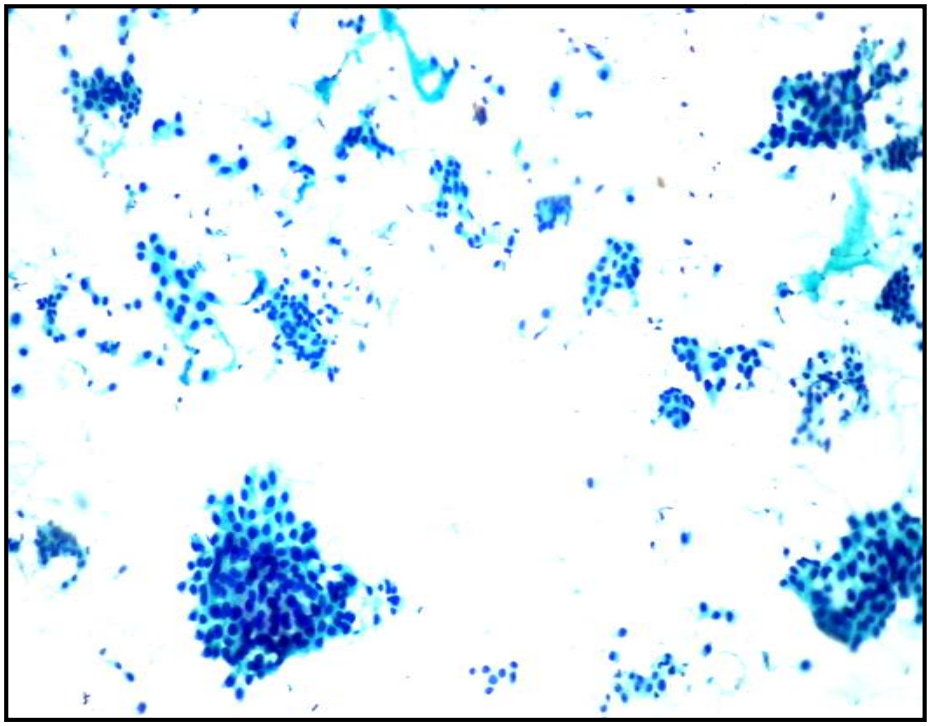


Fig 6: Malignant epithelial cells arranged singly and in clusters with mild pleomorphism (FNAC-Pap stain) 10X

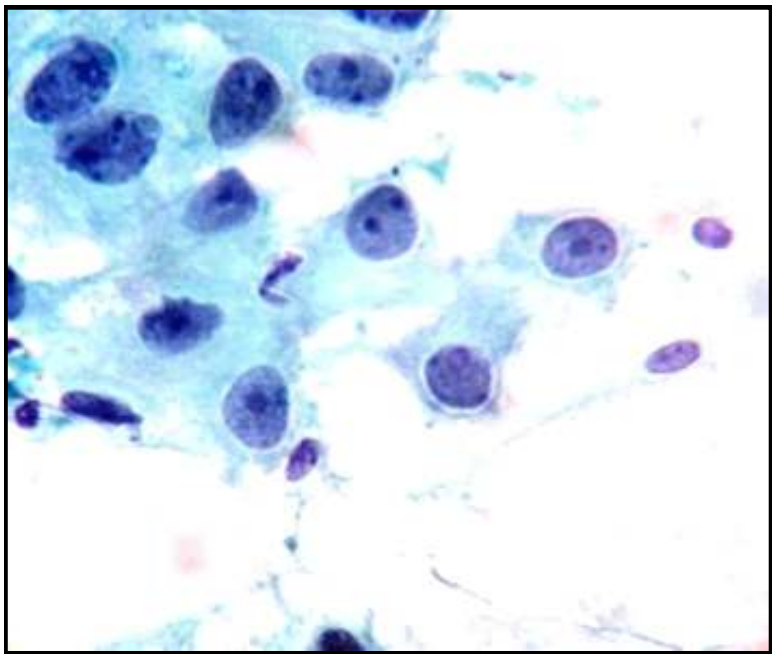


Fig 7: Noticeable nucleoli with granular chromatin (FNAC-Pap) 40 X

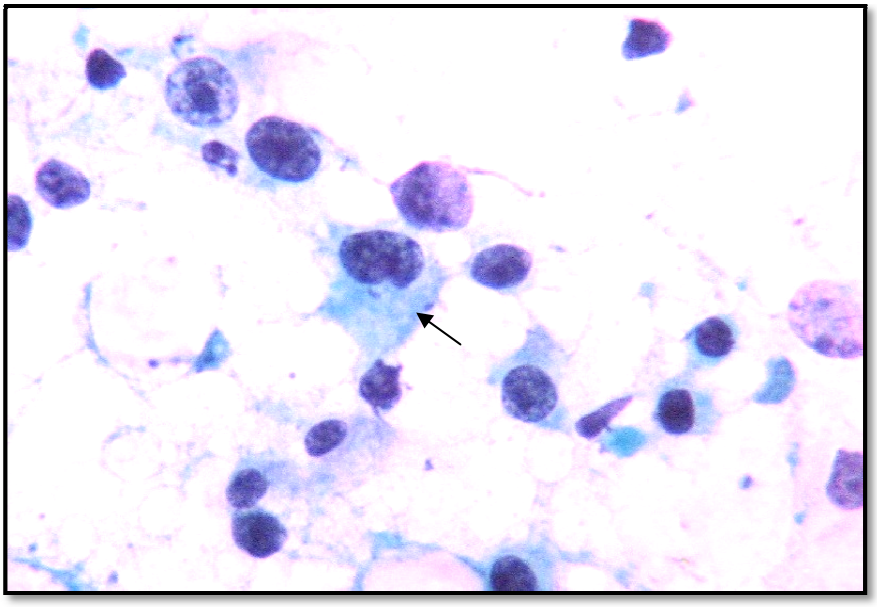


Fig 8: Nuclear membrane shows irregularity with folds (arrow)

(FNAC-Pap) 10X

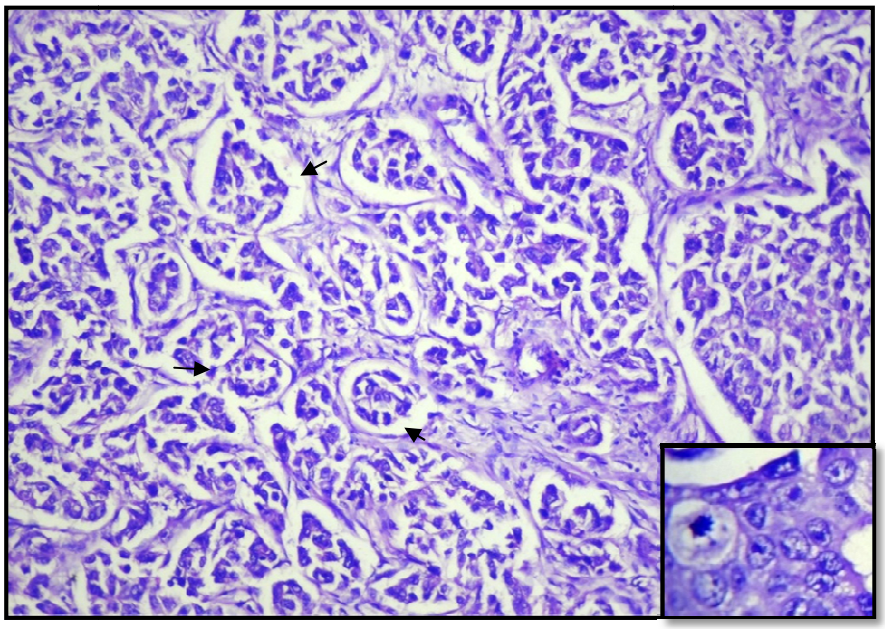


Fig 9: Tumor shows few tubules(arrow) in this field (HPE-H&E)10X.Inset shows vesicular nuclei and visible nucleoli with one mitotic figure

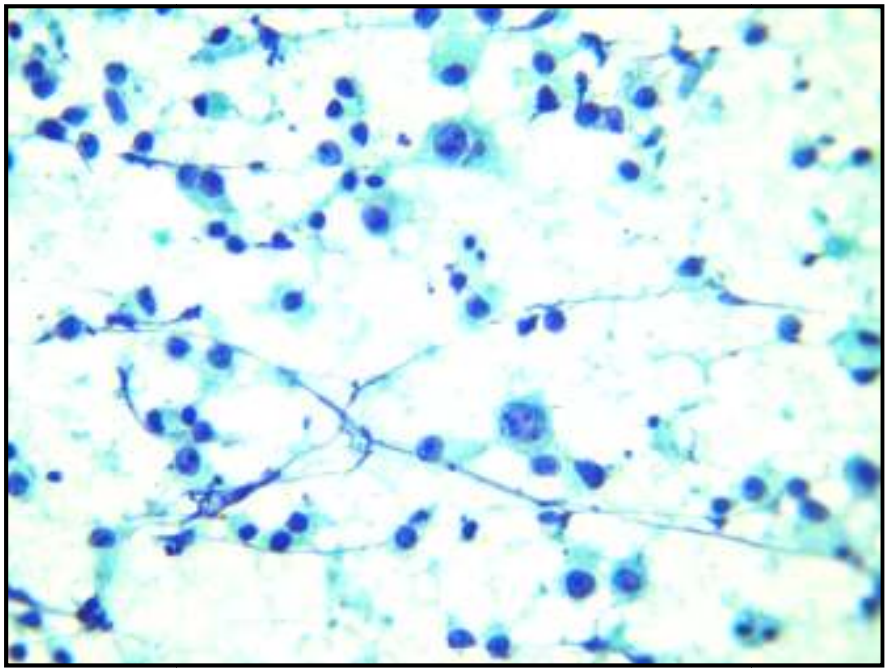


Fig 10:Malignant epithelial cells arranged singly

(FNAC-Pap stain)10X

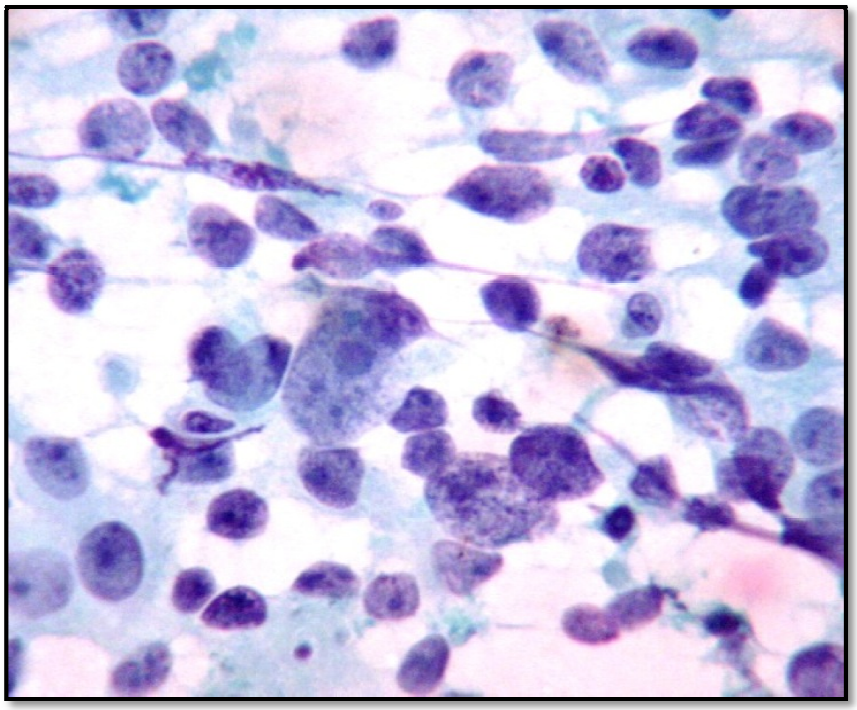


Fig 11: Marked nuclear pleomorphism with prominent nucleoli

(FNAC-Pap) 40X

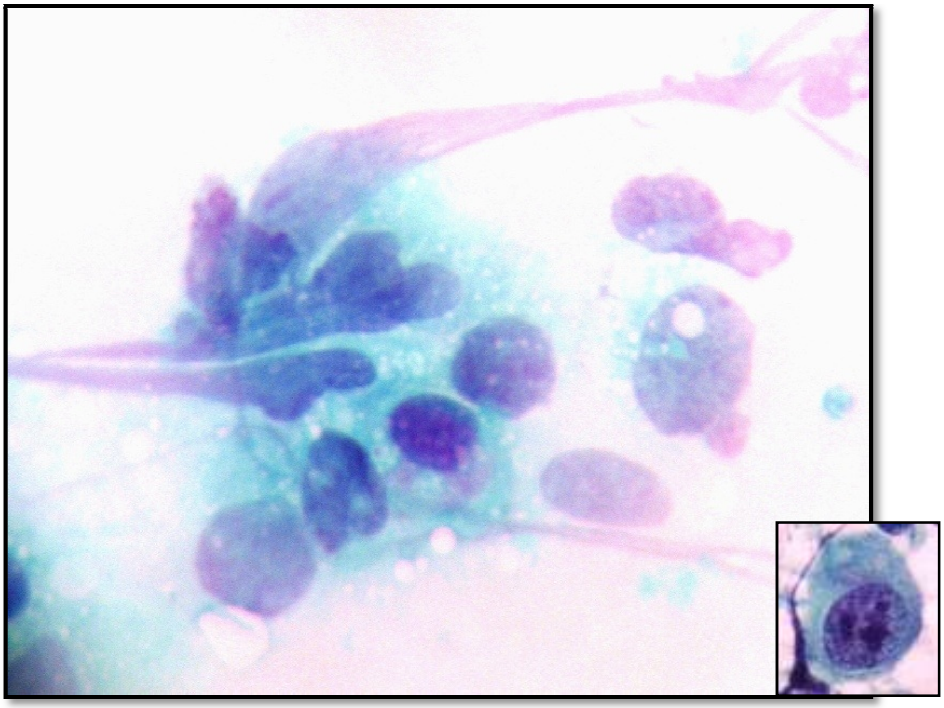


Fig 12:Nucleus showing bud and clefts. Inset shows clumped nuclear chromatin(FNAC-Pap stain) 40 X

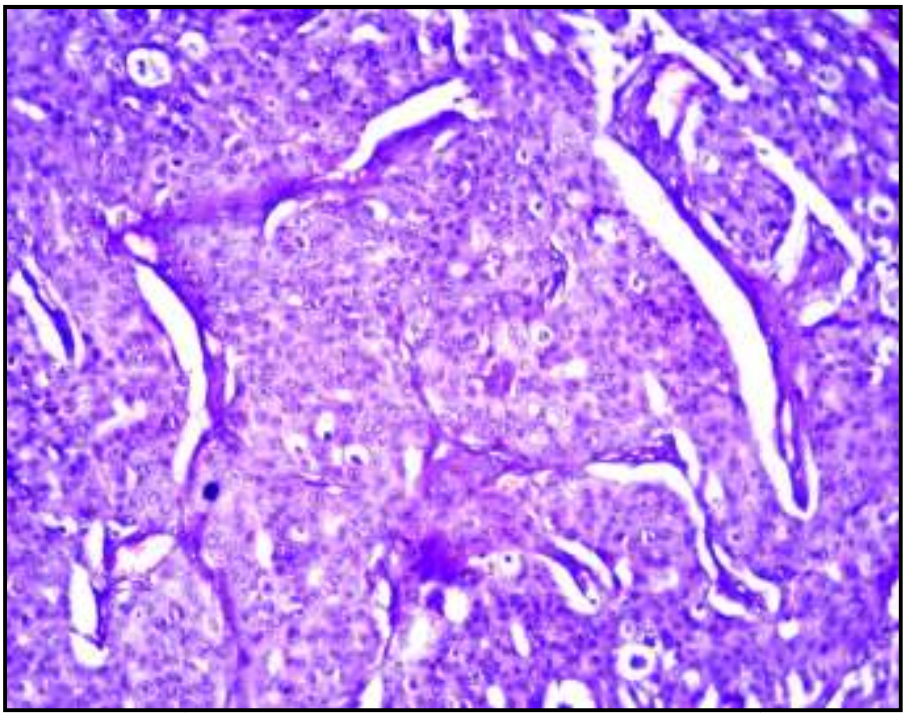


Fig 13:Tumor arranged in solid sheets(HPE-H&E) 10X

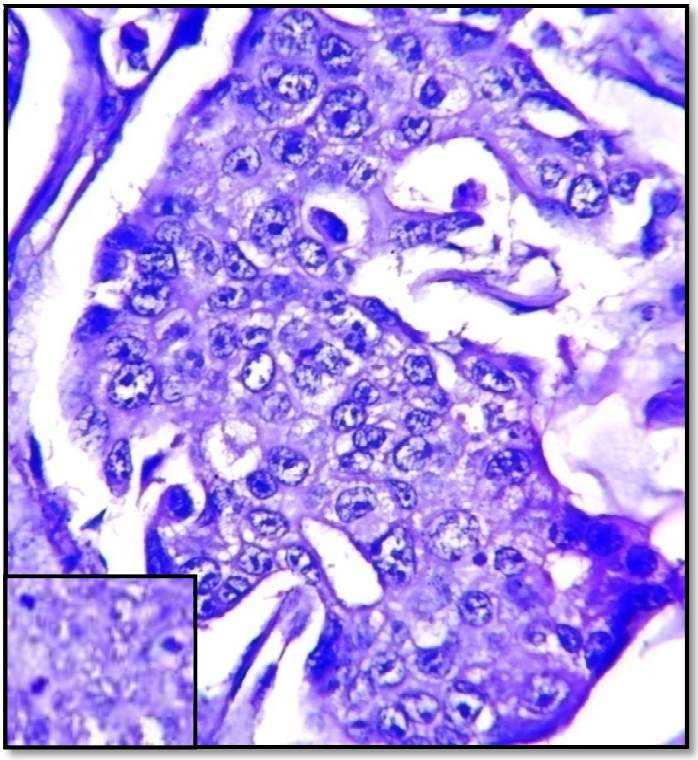


Fig 14:Large pleomorphic cells with prominent nucleoli. Inset shows three mitotic figures in one field (HPE-H&E) 40X

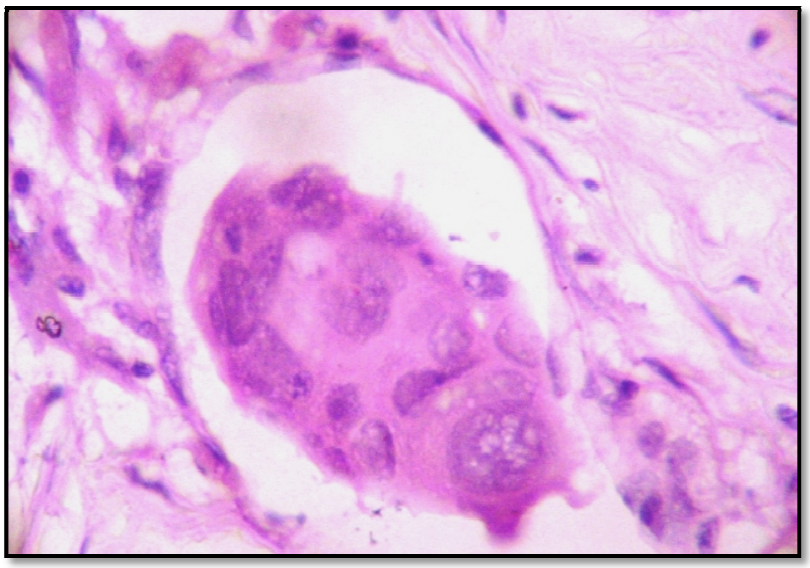


Fig 15: Lymphovascular invasion in one of the grade 3 tumor (HPE-H&E) 40X

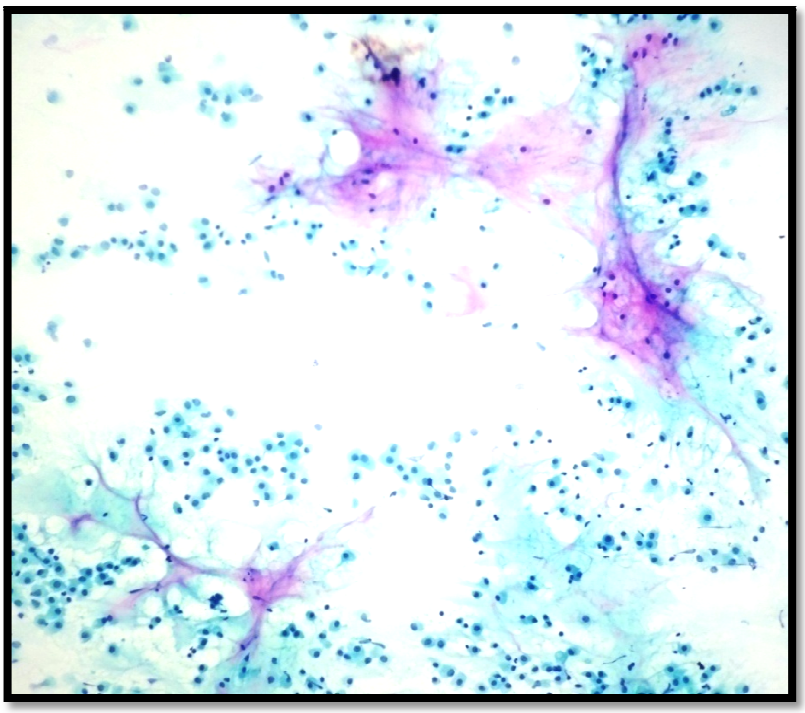


Fig 16: Tumor cells in a background of mucin (FNAC-Pap) 10X

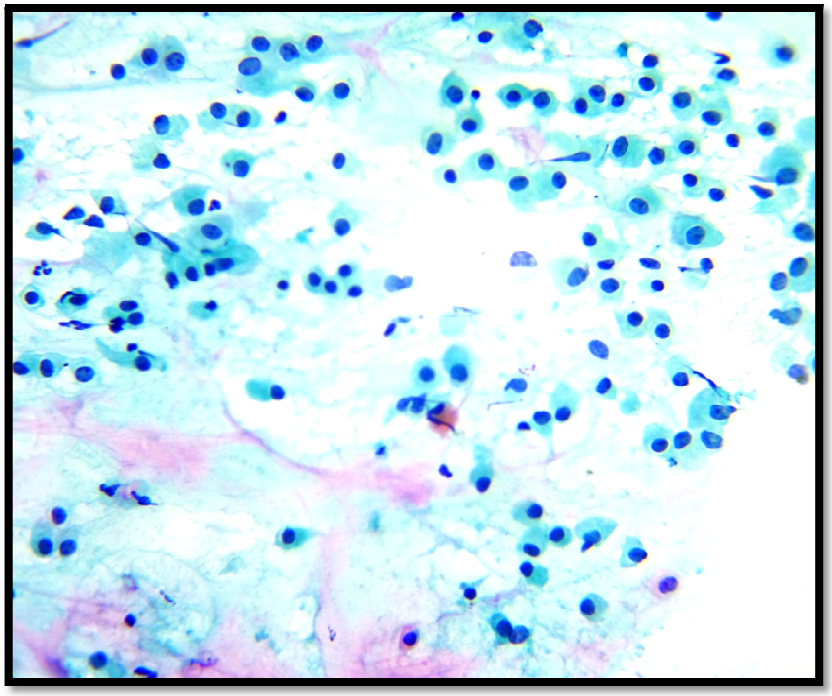


Fig 17:Cells arranged singly with moderate atypia in a background of mucin(FNAC-H&E) 40X

Fig 18: Gross - Well circumscribed mass with glistening cut surface

(HPE:186/12)

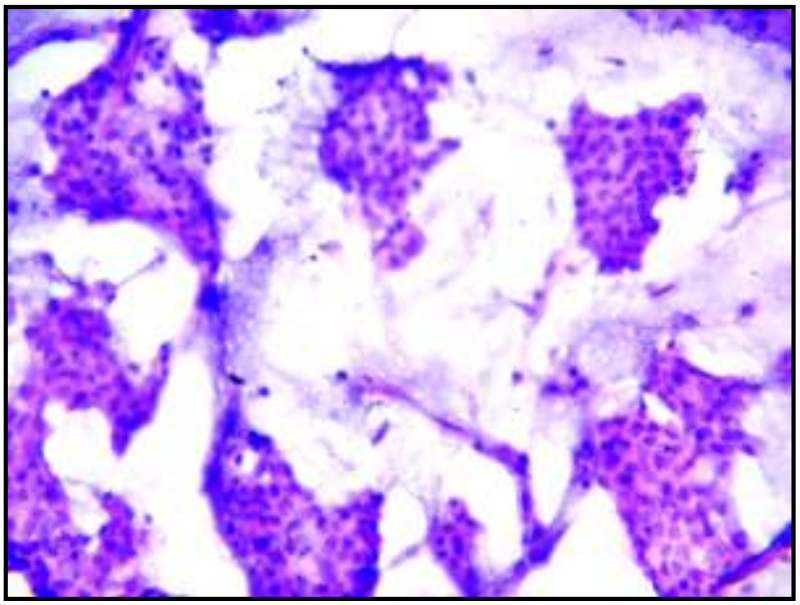


Fig 19: Nest of tumor cells in a pool of extracellular mucinwith small dark nuclei showing mild pleomorphism (HPE-H&E) 40X(HPE:186/12)

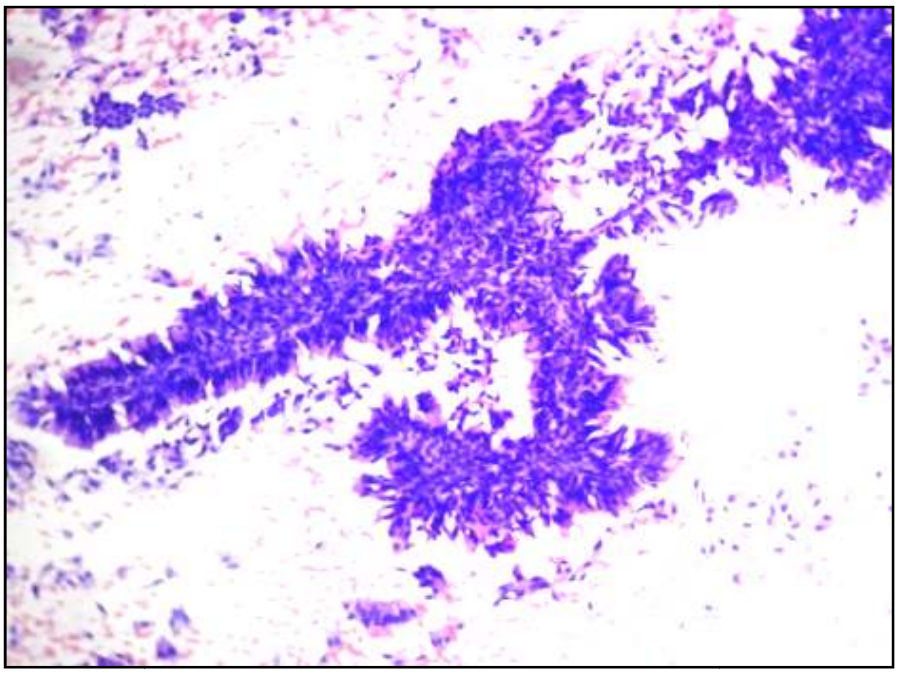


Fig 20: Branching papillae with fibrovascular core lined by atypical epithelial cells (FNAC-H&E)

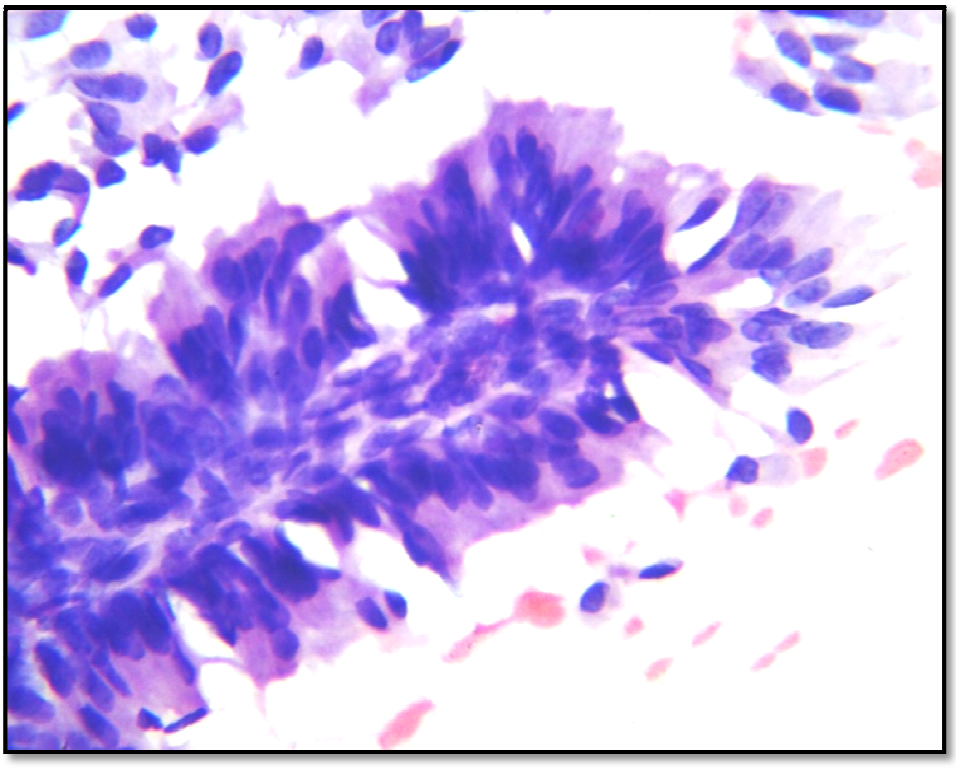


Fig 21: Papillae lined with tall columnar cells with nuclear stratification and atypia (FNAC-H&E)

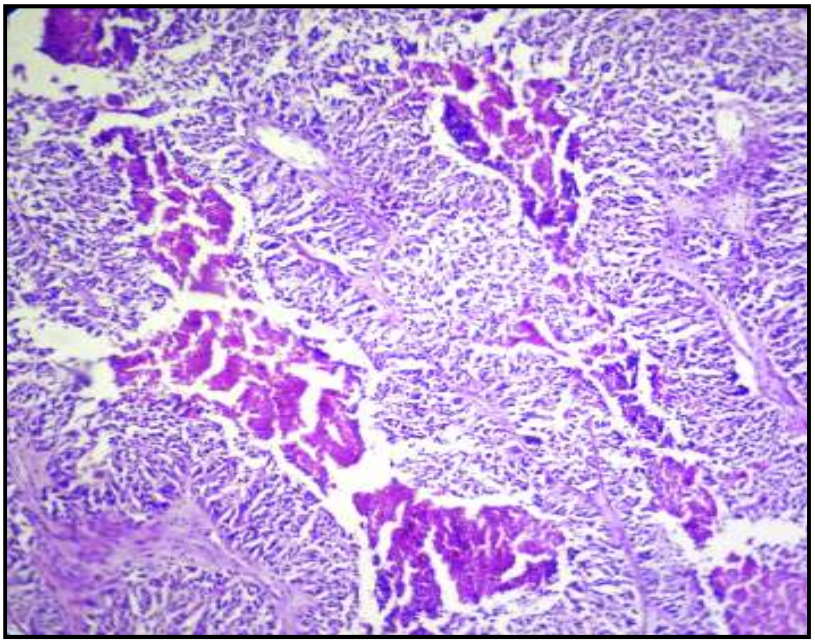


Fig 22: Papillary structures with fibrovascular core (HPE-H&E)10 X

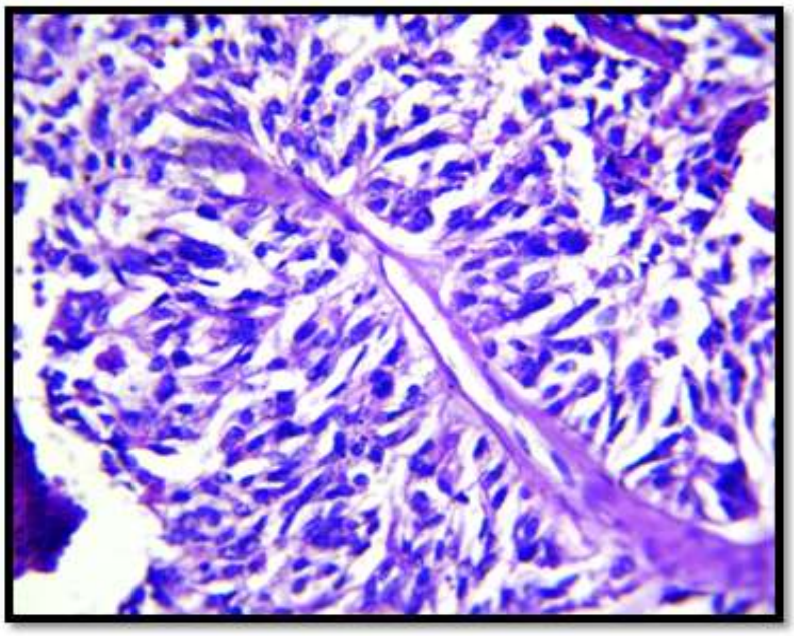


Fig 23: Papillae lined by multilayered atypical epithelial cells with moderate nuclear pleomorphism (HPE-H&E) 40X

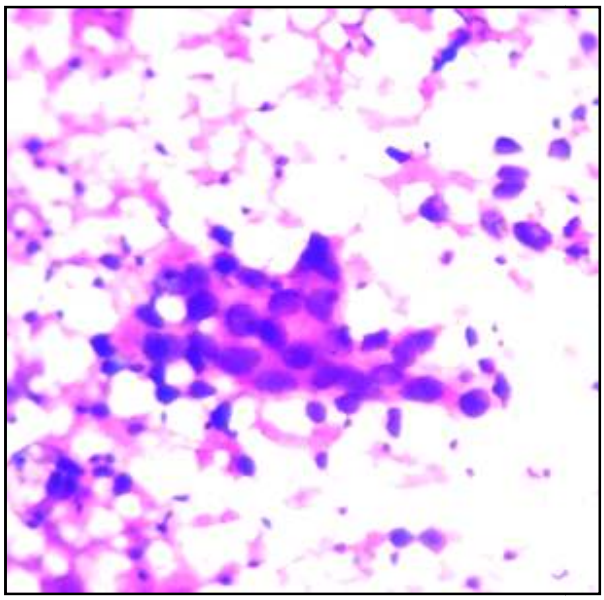


Fig 24: Malignant cell cluster with moderate pleomorphism in a background of scattered lymphocytes (FNAC-H&E) 40X

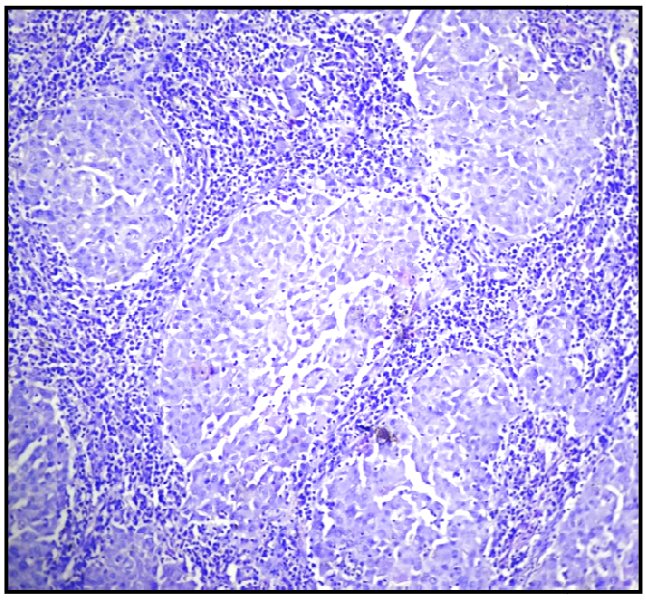


Fig 25:Syncytium of tumor cells surrounded by lymphoplasmacytic infiltrate(HPE- H&E) 10X

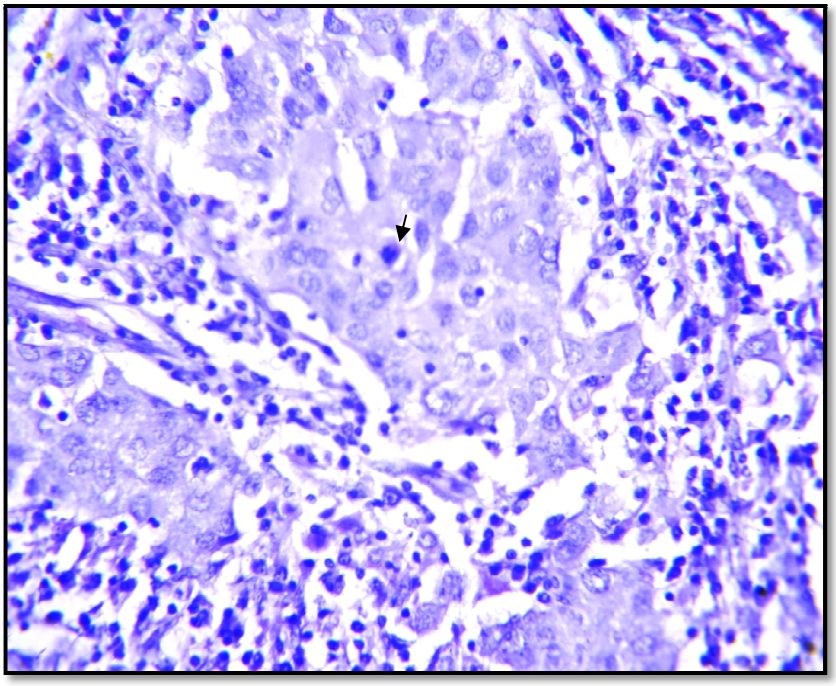


Fig 26:Synctium of tumor cells with moderate nuclear pleomorphism and single mitotic figure (arrow) (HPE-H&E) 40X

DISCUSSION

Breast carcinoma is a malignant disease with a variable prognosis. Assessment of prognostic parameters is of growing interest in recent days. The parameters consist of tumor size, lymph node status, [28]estrogen receptor status, [29] histological grading [27] and cell proliferation index [30.]All these features have been well studied on surgical specimens, however evaluation of prognostic factors and grading of cytologic samples have not been included in the routine practice. Cytologic grading is valuable, feasible and reproducible[26,16].This method also depicts the intrinsic characteristics of the tumor as well as its prognosis[20].

The need for prognostic grading in cytological samples of breast cancers is to identify rapidly growing tumors (grade III), which were more responsive to chemotherapy and better suited to pre-treatment with tamoxifen. Overtreatment of slow growing tumors was also reduced. Assessment of biological aggressiveness by cytological grading without removing the tumor would therefore be of immense value[15].

In this present study predominant tumors were grade 2 (46%) followed by grade1 (32%) and grade 3 (22%) in cytological samples. A study by Robles et al[67] showed a similar result, the predominant tumors were grade 2 (39%) followed by grade 1(36%) and then grade 3(25%). Studies done by Dash et al[14], Chabbra et al[57], Robinson et al[56] also

showed similar results. However in a study by Wani et al [18]grade 3 forms the second largest group as against to grade 1 in this study. This shows the increasing awareness and early presentation of the patients in our setup.

This study showed a high degree of concordance (86%)) between the cytological and histological grading systems. Khan et al[20] and Nijahawan et al[9] showed a concordance value of 84% and 82.5% respectively. A study by Milentijevic et al[7] also show a concordance value of 81%.High concordance value were also found by Taniguchi et al[16] Chabbra et al[57] and Bhargava et al[74] .Concordance between cytologic and histologic grading was observed more frequently in purely invasive carcinomas (85%)[6]. This study was close to these studies. However Dash et al[14], Moriquand et al and Gayathri et al[5] showed concordance of 77.5%.Sinha et al showed a concordance rate of 69.5% .

Low concordance in these studies was probably due to the inclusion of patients receiving chemotherapy and inclusion of cases diagnosed as atypical ductal hyperplasia and insitu carcinomas in cytological samples. In the present study the patients receiving neoadjuvant chemotherapy were excluded and only cases showing definitive features of ductal carcinoma in both cytology samples and

histologic specimens were included. This explains the high concordance between the grading systems.

In this study, association between cytological grading and histological grading system was highest among grade 2 tumors (91.3%) followed by grade 3(90.9%) and least with grade 1(75%). Sinha et al[4] states that grade 3 tumors showed a greater concordance. Jayaram et al[11] states that concordance was found to be higher among grade 3 tumors (83.3%) and low among grade 1 tumors (75%).These studies coincides with the present study. Khan et al[20] states that higher concordance was found among grade1 tumors(92.3%).Several studies states that concordance was high among high grade tumors. The present study also supports this view. Out of the seven discordant cases, four of grade 1 tumors were upgraded. Among two discordant grade 2 tumors, one was upgraded to grade 3 and other was downgraded to grade1.The one discordant grade 3 tumor was downgraded. The reason for the upgrading of tumors may be due to sampling errors in large size tumors and hetrogenous tumors. As orientation of the tumor cannot be made more accurate clinically, multiple passes in FNAC were aimed at the central portion of the tumor neglecting the active periphery. In histologic specimens the infiltrating edge of the tumor was correctly assessed and multiple blocks were made from that site to assess the grade. Another

reason of the upgrading of tumors may be due to inadequate samples. The low percentage of concordance in grade1tumors was due to the fact that these low grade tumors goes for a higher grade in the time lag between FNAC procedure and mastectomy,if there is long time gap between the two procedures. One of the reasons for downgrading of tumors may be due to the fact that only nuclear features were taken into consideration, while grading the cytologic samples. Tubule formation and mitotic count were not included. There may be subjective variability among the observers which explains the discrepencies.

Regarding the association between the cytological grade and lymph node status, node positivity was seen in 37.5%, 52.17% and 72.7% of grade 1, 2 and 3 tumors respectively. In a study by Khan et al[20] lymph node metastasis were seen in 15.4% of grade 1, 55.6% of grade 2 and 83.3% of grade 3 tumors. Most of tumors in the present study with more than four positive lymphnodes were grade 3 tumors.In a study by Robles et al [67]lymph node metastasis was observed in 8.3% of grade 1, 64.1% of grade 2 and 88% of grade 3 tumors. In a study by Dash et al[14] 74.2% of grade 3 tumors showed nodal metastasis in contrast to 27% in grade 1 tumors. Our study correlates more with the study by Dash et al[14].The association between the cytological grading and lymphnode status was not statistically significant ($p>0.05$).When this association had been

proved, level of resection can be assessed preoperatively by the cytological grade itself. But in this study cytologic grade did not correlate with axillary lymph nodes status .This is because in this study, criteria for the minimum number of nodes to be examined was not been defined. When the minimum number of nodes to be examined was increased to 10 nodes, better association can be obtained.

Other demographic factors like age and morphological factors like tumor size and tumor location were also studied .Regarding the age of the patient,most of the tumors were in the post-menopausal age group(52%).The mean age of presentation was 52.5 years. Most of grade 3 tumors were in the premenopausal age group accounting for 63.3% indicating its aggressiveness in the young age group. This evidence was supported by Pratap et al[3], Gann et al[1] and Gayathri et al[5].However the age of the patient did not correlate with the cytological grade statistically ($p>0.05$).

Regarding the tumor size majority of tumors (60%) were T_2 tumors (2-5cm, TNM staging) .Since the size of the tumor was an independent time dependent prognostic factor, an attempt was made to correlate tumor size with the cytological grade. No statistical significance was obtained ($p>0.05$).Similar results were observed in a study by Kim et al[28], Gann et al[1] and Gayathri et al[5].

Numerous studies have shown survival decreases with increasing tumor size and coincidental increase in axillary node positivity[29]. Roger et al reported significant distribution of frequency of axillary lymph node involvement in relation to tumor size. But in our study there is no significant correlation between tumor size and lymph node status($p>0.05$).

Regarding tumor location most of the tumors were located in outer quadrant accounting for 50%. However most of the grade 3 tumors were located in the central quadrant with 54.54%.A study by Harzah et al[15] shows that tumors in periareolar location are associated with poor prognosis. In the present study, tumors located in the inner quadrant (60%) show more lymph node positivity than other quadrant tumors.This is in contrast to all other studies where inner quadrant tumors show lower rate of lymph node positivity[1,5]. Similar to the present study Reger et al also showed no correlation between the tumor location and lymh node status.

SUMMARY

Breast carcinoma is the second most common cause of cancer related deaths among female next to cervical cancer. In some cities, the incidence of breast cancer has been increasing. Invasive duct carcinoma is the most common malignancy of breast, accounting for 70-80%. Cytological evaluation of aspirates for early diagnosis and prognostication of breast malignancies is important to study the intrinsic feature and biologic aggressiveness of the tumor while the tumor is still in vivo. Among the various prognostic factors, nuclear grading is the most feasible, cost effective and can be done if proper staining techniques and qualified pathologist are available. No sophisticated instruments and laborious work is needed.

As FNAC is done as a baseline investigation that can be done on outpatient basis, a number of studies have attempted to grade the cytological samples. Cytologic grading of breast cancer is not well established despite histologic grading having gained a strong foothold. So attempt had been made to correlate the cytologic grading with well established Nottingham modification of Scarff Bloom Richardson grading. The cytological grade was also correlated with other prognostic factors like age,tumor size and lymph node status.

A study was done on fifty cases of ductal carcinoma where cytological diagnosis of Invasive ductal carcinoma was confirmed by histology. Pateints who had underwent preoperative neo adjuvant chemotherapy was excluded . Cytological samples were stained using H&E stain and Papanicolaou stain. Cytologic grading was done by Robinson method and compared with histologic grading system .The conclusion made were

1. Most of the tumors were grade 2 tumors(46%)
2. The concordance between cytological grade and histological grade was 86%, nearly 43 out of 50 cases
3. The remaining 7 cases (14%) showed discrepancy. Among these cases five were upgraded and two were downgraded.
4. The association between cytological grade and histological grade were statistically significant ($p<0.05$)
5. Most common age of presentation was postmenopausal age group (52%)
6. However the grade 3 tumors was high among premenopausal age Group (63.3%)

7. The association between the age of the patient and the cytological grade was not statistically significant($p>0.05$)

8. The Lymph node positivity was noted among 37.5%, 52.17%, 72.7% of grade 1, 2 and 3 tumors respectively. High grade tumors show increased nodal positivity.

9. The association between cytological grade and lymphnode status was not statistically significant ($p>0.05$)

10. Most of the tumors were T2 (2-5 cm).The association between tumor size and cytologic grade was not statistically significant ($p>0.05$).The association between tumor size and lymph node status was also not significant($p>0.05$)

11. Most of the tumors were located in outer quadrant 50%.most of grade 3 tumors were located in the central quadrant. Tumors located in the inner quadrant show a increased nodal postivity(60%)

This concludes that cytological grading should be included in all FNAC reports of ductal carcinoma of breast, so that appropriate decision can be made regarding the neoadjuvant chemotherapy can be taken and overtreatment of low grade carcinomas can be avoided. Preoperative

grading of breast cancer helps the surgeons to plan the type of operation and level of lymph node resection. This suggest the use of nuclear morphometry to increase the accuracy and reduce the observer variability.

CONCLUSION

Due to the Increasing incidence of breast cancer in recent years, the role of FNAC in the early identification of breast cancer has been tremendously increased. This present study evaluates prognostic factors in cytological samples .Of the various prognostic factors, cytological grading is the one which is more feasible and reproducible.The histological grade is one of the well known morphological prognostic factor and had been included in all reports of breast malignancies. This study shows a significant correlation between the cytologic grade and histologic grade. This indicates that cytological grade predicts the tumor aggressiveness.

So it was concluded that the cytological grading should be included in all FNAC reports. So that appropriate decision regarding the preoperative neoadjuvant chemotherapy can be made and overtreatment of low grade cancers has been avoided. In this study cytological grading did not correlate with the lymphnode status .When this correlation has been proved ,the level of lymph node resection can be planned preoperatively .Inspite of adequate efforts made to reduce the distortion in estimates, there is some degree of measurement bias and interobserver variability in this grading system.

This present study suggests the use of ancillary techniques like nuclear morphometry, immunohistochemistry for hormone receptors and proliferative indices done on cytological samples for further evaluation the tumor biology and aggressiveness.

BIBLIOGRAPHY

1. Kim Y B,Hwang T S et al .correlation of tumor grade and other prognostic factors in Invasive breast carcinoma.Journal of Korean Cancer Association1994;26(6):926-35

2.Rosai and Ackerman's surgical pathology ,Elsevier 9th edition vol 2:1802- 1804

3.Pratap R, Shousha et al.Breast carcinoma in women under the age of 50 years:Relationship between p53 immunoreactivity,tumor grade and lymph node status.Breast research and treatment1998;49:35-39

4.Sinha SK, Sinha N, Bandyopadhyay R, Mondal SK. Robinson's cytological grading on aspirates of breast carcinoma: Correlation with Bloom Richardson's histological grading. J Cytol 2009;26:140-3

5.R.Gayathri et al Cytohistological grading of invasive ductal carcinoma breast with coorelation of lymph node status RGUHS; 2010:1-11

6.Brifford et al,Le Dowssal V et al. Cytologic nuclear grading of fine needle cytopunctures of breast carcinoma. Comparison with histologic nuclear grading and image cytometric data.Anal Quant Cytol Histol,1997 Apr;19(2):114-22.

7.Milentijevi A et al. Cytological grading of breast carcinoma with histological correlation J BUON 2005 Apr-Jun;10 (2):251-6.

8. Silverman JF et al Diagnostic accuracy cost effectiveness and triage role of fine needle aspiration biopsy in the diagnosis of palpable breast lesions 1995,1:3-8

9. Khan N,Rana F,Afroz N et al ,cytomorphplogical grading of breast carcinoma with special reference to apoptotic rates and lymph node metastasis Ind J Pathol Microbiol 2007;50(3):613-18

10.Guidelines for non operative diagnostic procedures and reporting in breast cancer screening.Non operative Diagnosis subgroup of National coordinating group for breast screening pathology NHSBSP publication 2001;50:15

11.Jayaram G Elsayed EM .Cytologic evaluation of prognostic markers in breast carcinoma.Acta Cytological 2005;49:605-10

12.Khan MZ et al ,cytopothological grading as a predictor of histopathological grade in ductal carcinoma (nos) of breast on air dried Diff quick smears.Diagnostic cytopathology .2003;29185-93.

13Abati A, Abele J Baus et al .The uniform approach to breast fine needle aspiration biopsy;Diagnostic cytopathlogy.1997;16:295-391

14 Dash A, Mohanty R et al .Aspiration smear pattern as a predictor of biologic behavior in breast carcinoma J Cytol 2005;22(1):19-21

15. HarzahP,Dhir M et al.Prognostic significance of location of primary tumor in operable breast cancer.Indian Journal Cancer 2009;46(2):139-145

16 Taniguchi E, Yang Q, Tang W, Nakamura Y, Shan L, Nakamura M, *et al* .Cytologicalgrading of invasive breast carcinoma: Correlation with clinicopathologic variables and predictive value of nodal metastasis. Acta

Cytol 2000;44:587-91.

. 17.A.K.Das, Kusma kapila et al. Comparative evaluation of grading of breast carcinomas in fine needle aspirates by two methods. Indian J Med Res 118 2003 247-50

18.Wani FA, Bhardwaj S, Kumar D, Katoch P. Cytological grading of breast cancers andcomparative evaluation of two grading systems. J Cytol 2010; 27:55-8

19.TS Rekha NM Nandhini et al. Validity of different cytological grading systems of Breast carcinoma-a hospital base study in south india.Asian Pacific J cancer Prev 2011;12:3013-16

20. Khan N, Afroz N, Rana F, Khan MA. Role of cytologic grading in prognostication of Invasive breast carcinoma. J Cytol 2009;26:65-8

21.Idris et al .Comparison of efficacy of three stains used for detection of cytologic changes in Sudanese females with breast lumps;Sudanese Journal of public health2009;4:275-77

22.Sistrunk WE ,Mac Carty WC.Lifeexpentncy following radical amputation for carcinoma of breast. A clinical and pathologic study of 218 cases.Ann Surgey1922;75:61-69

23.Heimann R,Hellman S.Clinical progression of breast cancer malignant behavior:what to expect and whento expect itJ Clin Oncol 2000;18:591-599

24.Blamey RW,Elston CW,Ellis IO.When is a patient cured of breast cancer?Mod Pathol 2000 :13-18 A

25. Rosen PP.The pathological claasification of human mammary carcinoma:Past, present and future.Ann Clin Lab Sci 1979;9:144-56

26. Robinson IA, McKee G. Cytological grading of breast carcinoma. Acta Cytol 1995;39:1257.

27. Elston C, Ellis I. Pathological prognostic factors in breast cancer: I, The value of histological grade in breast cancer: Experience from a large study with long-term follow-up. Histopathology 1991;19:403-10.

28. Kim Y B,Hwang T S et al .correlation of tumor grade and other prognostic factors in Invasive breast carcinoma.Journal of Korean Cancer Association1994;26(6):926-35

29.Say CC,Donegan WL.Invasive carcinoma of the breast:Prognostic significance of tumor size and involved axillary lymph nodes.Cncer 1974;34:468-471

30.Weaver DL, Rosenberg RD et al.Pathologic findings from the breast cancer consotorium.Population basd outcomes in women undergoing biopsy after screening mammography.Cancer 2006;106:732-742

31.Quiet CA,Ferguson DJ et al.Natural history of node positive breast cancer:the curability of small cancer with limited number of positive nodes.J Clin Oncol1996;14:3105-3111

32. Sinha SK, Sinha N, Bandyopadhyay R, Mondal SK. Robinson's cytological grading on aspirates of breast carcinoma: Correlation with Bloom Richardson's histological grading. J Cytol 2009;26:140-3

33.Haybittle J L,Blamey R W,Elston C W et al 1982 A Prognostic index in primary breast cancer.Br J Cancer 45:361-366

34.Todd J H,Dowle C ,William M R et al .Confirmation of prognostic index of primary breast Cancer Br J Cancer 56:489-92

35.Elston C W,Gresham G A,Rao G S et al .The cancer Research Campaign Trial for EarlBreast cancer-pathological aspects,Br J Cancer 1982 45:665-69

36.Fisher E R, Palekar A S et al.Pathologic findings from the National Surgical adjuvant Breast Project .Am J Clin Pathol ,1980;73:313-22.

37.NematoT,Vana J et al .Management and survival of female breast cancer:results of a national survey by the American college of surgeons.cancer 1980;45:2917-24.

38.Haagensan C D1986 Diseases of breast.W B Saunders,Philadelphia

39.Schottenfield D,Nash A G et al.Ten year results of the treatment of primary operable breast Carcinoma 1976;38:1001-7.

40.Jean .F.Simpson Robert gray et al.Prognostic value of Histologic grade and proliferativeactivity in Axillary Node- Positive breast cancer:Resultsnfrom the eastern cooperative oncology group companion study,Journal of clinical oncology 2000;18:2059-69

41.Gold RH,Main G,Zippin C et al.Infiltration of mammary carcinoma as a indicator of axillary metastasis.A Preliminary report.Cancer 1972;29:35-40

42.Carter D,Pipkin RD,Shepard RH et al.Relationship of necrosis and tumor border to lymph node metastasis and 10 year survival in carcinoma of the breast.Am J surg 1978;2:39-46

43. Contesso G, Mouriesse H, Friedman S, et al: The importance of histologic grade in long-term prognosis of breast cancer: A study of 1,010

patients, uniformly treated at the Institute Gustave-Roussy. J Clin Oncol ,1987;5:1378-1386.

44. Dalton LW, Page DL, Dupont WD: Histological grading of breast cancer: A reproducibilitystudy. Cancer 73:2765-2770, 1994

45.John .S.Meyer ,Consulo Alvariz Breast carcinoma malignancy grading by Bloom–Richardsonsystem *vs* proliferation index: reproducibility of grade and advantages of proliferation index;Modern Pathology 2005; 18: 1067–1078

46.Pinder SE,Murray S,Ellis IO et al.The importaance of histologic grade of invasive breast Carcinoma and response to chemotherapy.Cancer 1998;83:1529-1539

47. Consensus statement: Treatment of early-stage breast cancer—National Institutes of HealthConsensus Development Panel. J Natl Cancer Inst Monogr ,1992;11:1-5

48.Davis BW,Gelber RD et al.Prognostic significance of tumor grade in clinical trials of adjuvant therapy for breast cancer withaxillary lymph node metastasis.Cancer 1986;58:2662-2670

49.Masters JRW, Millis RR et al.Response to endocrine therapy and breast cancer differentiation.Breast Cancer Res Treat1986;7;31-34

50.Williams MR,BlameyRW et al.Histologic grade in predicting response treatment to endocrine Therapy. Breast Cancer Res Treat1986;8:165-166

51.Strenberg, Stacey .E.Mills,Darryl Carter et al.strenberg's Dignostic surgical pathology.fifth edition;vol 1:285-345

52.Fritz rank et al, MD,Per Dombernowsky et al .Histologic Malignancy Grading of Invasive Ductal Breast Carcinoma Cancer 1987;60:1299-1305,

53.Parham DM,Hagen N,Brown RA et al.Simplified method of grading primary carcinomas of the breast.J Clin Pathol 1992;45:517-520

54.van Deist PJ,Baak JPA,Matze-cok P et al.Reproducibility of mitosis counting in 2,469 breast cancer specimens;result from multicentric Morphometric Mammary carcinoma Project.Human Pathol 1992;23:603-607

55.Kupio T,Collan T.Still more about counting mitoses.Human Pathol1996;27:1110-1111

56.Robinson IA, McKee G, Nicholson A, D'Acry J, Jackson PA, Cook MG, et al. Prognostic value of cytological grading of fine-needle aspirates from breast carcinomas. Lancet 1994;343:947-9.

57. Chhabra S, Singh PK, Agarwal A, Singh SN, Bhagoliwal A. Cytological grading of breast carcinoma - a regression analysis. J Cytol 2005;22:62-5

58.Frances P.O'Malley,Sarah .E.Pinder,Anna Marie Mulligan Breast Pathology:Foundations in Diagnostic pathology second edition;260-271

59.Kuerer HM,Neuman LA,Smith TL et al.Clinical course of breast cancer patients with complete pathologic primary tumor and axillary lymph node response to Doxorubin-based neoadjuvant chemotherapy J Clin1999;17:460-469

60.Rajan R,Poniecka A,Smith TL et al.Changes in tumor cellilarity of breast carcinoma after neoadjuvant chemotherapy as a variable in pathologic assessment of response.Cancer 2004;100:1365-1373

61.Brifford M,Spyratos F,Tubaina-Huhn M et al.Sequential cytopunctures during preoperative for primary breast cancer.Cancer1989;63:631-637

62.Freirson Jr HF,Fechner RE.Histologic grade of locally advanced infiltrating ductal carcinoma after treatment with induction chemotherapy.Am J Clin Pathol 1994;102:154-157

63.Addington S,Sharkey L,Fowler C et al.Effects of preoperative chemotherapy on mrpholog of resectable breast carcinoma.Lab invest 1994;70:12A

64.Locker A P,Ellis I O et al.Factors influencing the local reccurence after excision and chemotherapy for primary breast cancer.Br J Surg 1989,76;890-94

65.O'Rourke S,Galea M H et al.An audit of local recurrence after simple mastectomy.Br J Surg.1994,81;386-89

66. Lester SC. The breast. In: Robbins and Cotran, Pathologic basis of disease. Kumar V, Abbas ,A K, Fausto N, editors. 7 th ed. Philadelphia: WB Saunders Company Elsevier Inc; 2004. p. 1129-31.

67. Robles Frvas A, Gonzαlez-Cαmpora R, Martvnez-Parra D, Robles-Frvas MJ, Vαzquez-Cerezuela T, Otal-Salaverri C, *et al*. Robinson cytologic grading of invasive ductal breast carcinoma: Correlation with histologic grading and regional lymph node metastasis. Acta Cytol 2005;4:149-53.

68.Masood.S. Prognostic factors in breast cancer: Use of cytologic preparations. Diagn Cytopathol 1995;13:388-95.

69. Bozzetti C, Nizzoli R, Naldi N, Guazzi A, Camisa R, Manotti L, *et al*. Nuclear grading and flow cytometric DNA pattern in fine-needle aspirates of primary breast cancer. Diagn Cytopathol ,1996;15:116-20.

70. Blamey R W,Davies C J,Elston C W et al.Prognostic factors in breast cancer:the formation of prognostic index.Clin Oncol 5:22

71.Paul Peter Rosen.Rosen's Breast pathology.third edition.358-405

72.National coordinating committee for breast screening pathology. Pathology reporting in breast cancer screening 2nd edition sheffeild:NHS-BSP publications1995

73.Rippey JJ .More about counting mitoses .Human Pathol 1996;27:1109-1110

74.Bhargava V, Jain M, Agarwal K, Thomas S, Singh S. Critical appraisal of cytological nuclear grading in carcinoma of the breast and its correlation with ER/PR expression. J Cytol 2008;25:58-61

ANNEXURE-I

PROFOMA

Name: Age:

Ward: IP/OP No:

Address:

Presenting complaints:

- Breast lump
- Pain
- Nipple discharge
- Skin ulceration

Duration of presenting complaint:

Past history:

- History of previous surgeries for breast lump
- History of chemotherapy/Radiotherapy
- History of breast lump in other breast

Family history:

Personal history

- Diet
- Menstural history
- Breast feeding history

General examination

Nourishment : Built: Consious:

Pallor: Jaundice: Cyanosis: Clubbing:

PR: RR: BP: Febrile/afebrile:

Lymphadenopathy: Edema:

Local examination of the breast

Side:Right/Left

Quadrant:upper/outer/inner/lower/cental

Size of the tumor:

Fixity to skin:yes/no Fixity to underlying fascia:yes/no

Examination of axillary lymph nodes:

Number of nodes:

Mobile/fixed:

Size:

Group of nodes:Anterior/posterior/lateral/apical

Cytological diagnosis:

Any special type:yes/no

Cytological grading

Cell Dissociation:1/2/3

Nuclear size: 1/2/3

Cell uniformity: 1/2/3

Nucleoli: 1/2/3

Nuclear margin: 1/2/3

Chromatin pattern:1/2/3

Cytological grade:I/II/III

Gross examination of modified radical mastectomy specimen

Size of the specimen including skin ,nipple &areola:

Size of the tumor:<2cm/2-5cm/>5cm

Margins:infiltrative/circumscribed

Quadrant:outer/inner/central

Histologic diagnosis:

Any special type:

Lymph node status:no:of positive nodes/no:of total nodes examined

Histologic grading

Tubule formation:1/2/3

Nuclear pleomorphism:1/2/3

Mitotis:1/2/3

Histoloigic grade:I/II/III

ANNEXURE-II

CONSENT FORM

The doctor has explained that, I (name of the patient)……., have a lump in my……(side) breast/s and that FNAC is proposed for the purpose of diagnosis. I understand the complications and thereby I agree for this procedure.

SIGNATURE

ANNEXURE-III

POINTS FOR MITOTIC COUNTS CORRESPONDING TO MICROSCOPC FIELD AREA.

FIELD DIAMETER	NUMBER OF MITOSIS		
	SCORE 1	SCORE 2	SCORE 3
0.4	upto 4	5to8	9or more
0.41	upto 4	5to9	10 or more
0.42	upto 4	5 to 9	10 or more
0.43	upto 4	5 to 10	11 or more
0.44	upto 5	6 to 10	11or more
0.45	upto 5	6 to11	12 or more
0.46	upto 5	6 to 11	12 or more
0.47	upto 5	6 to 12	13 or more
0.48	upto 6	7 to12	13 0r more
0.49	upto 6	7 to13	14 or more
0.5	upto 6	7 to 13	14 or more
0.51	upto 6	7 to14	15 or more
0.52	upto 7	8 to 14	15 or more
0.53	upto 7	8 to15	16 or more
0.54	upto 7	8 to 16	17 or more
0.55	upto 8	9 to 16	17 or more
0.56	upto 8	9 to 17	18 or more
0.57	upto 8	9 to 17	18 or more
0.58	upto 9	10 to 18	19 or more
0.59	upto 9	10 to 19	20 or more
0.6	upto 9	10 to 19	20 or more
0.61	upto 9	10 to20	21 or more
0.62	upto10	11 to21	22 or more
0.63	upto 10	11 to21	22 or more
0.64	upto11	12 to22	23 or more
0.65	upto 11	12 to23	24 or more
0.66	upto 11	12 to 24	25 or more
0.67	upto 12	13 to25	26 or more
0.68	upto 12	13 to 25	26 or more
0.69	upto 12	13 to26	27 or more
0.7	upto 13	14 to 27	28 or more

S.NO	HPE NO	FNAC NO	AGE	TUMOR SIZE	TUMOR LOCATION	CYTOLOGIC DIAGNOSIS	CYTOLOGIC GRADE	HISTOLOGIC DIAGNOSIS	HISTOLOGIC GRADE	LYMPHNODE STATUS
1	2285/11	F1003/11	65	6.5cm	Outer	DC	Grade1	IDC NOS	Grade 2	0
2	2696/11	FI318/11	54	7cm	Outer		Grade 2	IDC NOS	Grade 2	2
3	2697/11	F1944/11	40	3.5cm	Central	DC with lymphocytes	Grade 3	Atypical medullary carcinoma	Grade 3	0
4	2768/11	F1984/11	55	2cm	Outer	DC	Grade 2	IDC NOS	Grade 2	2
5	2809/11	F2019/11	70	2.5cm	Outer		Grade 1	IDC NOS	Grade 1	5
6	2859/11	F1087/11	60	7cm	Outer	DC	Grade 3	IDC NOS	Grade 3	4
7	2866/11	F851/11	65	6.5cm	Inner	DC	Grade 2	IDC NOS	Grade 2	8
8	2895/11	F2020/11	70	5cm	Outer	DC	Grade 1	IDC NOS	Grade 1	0
9	2075/11	F1121/11	44	6cm	Inner	DC	Grade 1	IDC NOS	Grade 3	5
10	2188/11	F1630/11	47	4cm	Inner	DC	Grade 1	IDC NOS	Grade 1	0
11	1994/11	F1491/11	45	3cm	Central	DC	Grade 1	IDC NOS	Grade 1	0
12	1978/11	F1329/11	44	3.5cm	Outer	DC	Grade 2	IDC NOS	Grade 2	1
13	1977/11	F159/11	56	3cm	Outer	DC	Grade 2	IDC NOS	Grade 2	0
14	1942/11	F1442/11	45	4cm	Outer	DC	Grade 1	IDC NOS	Grade 1	0
15	2269/11	F1618/11	32	3.5cm	Central	DC	Grade 3	IDC NOS	Grade 3	0
16	2923/11	F1316/11	49	3.5cm	Central	DC	Grade 1	IDC NOS	Grade 2	4
17	12-Mar	F1764/11	42	3cm	Outer	DC	Grade 3	IDC NOS	Grade 3	8
18	43/12	F2145/12	52	2.2cm	Outer	DC	Grade 1	IDC NOS	Grade 3	0
19	75/12	F03/12	46	4.2cm	Outer	DC	Grade 1	IDC NOS	Grade 1	0
20	967/12	F1158/12	55	1.7cm	Inner	Papillary carcinoma	Grade 2	Papillary carcinoma	Grade 2	0

21	170/12	F2264/12	65	1.8cm	Outer	DC	Grade 2	IDC NOS	Grade 2	1
22	186/12	F87/12	58	10cm	Outer	Mucinous carcinoma	Grade 1	Mucinous carcinoma	Grade 1	0
23	237/12	F114/12	51	7cm	Outer	DC	Grade 2	IDC NOS	Grade 3	6
24	253/12	F126/12	40	7.5cm	Outer	DC	Grade 3	IDC NOS	Grade 3	7
25	304/12	F204/12	55	3cm	Inner	DC	Grade 2	IDC NOS	Grade 2	2
26	321/12	F100/12	59	1.9cm	Outer	DC	Grade 1	IDC NOS	Grade 1	0
27	435/12	F136/12	38	2cm	Outer	DC	Grade 2	IDC NOS	Grade 1	4
28	436/12	F1923/11	65	8cm	Outer	DC	Grade 1	IDC NOS	Grade 1	0
29	453/12	F120/12	46	5cm	Central	DC	Grade2	IDC NOS	Grade 2	0
30	477/12	F886/12	56	8.5cm	central	DC	Grade 3	IDC NOS	Grade 3	3
31	536/12	F125/12	40	1.5cm	Inner	DC	Grade 2	IDC NOS	Grade 2	0
32	547/12	F2030/11	53	5.3 cm	Outer	DC	Grade 2	IDC NOS	Grade 2	5
33	548/12	F248/12	56	3cm	central	DC	Grade 2	IDC NOS	Grade 2	3
34	570/12	F78/12	39	8.5cm	central	DC	Grade 3	IDC NOS	Grade 3	18
35	584/12	F264/12	43	8.2cm	Central	DC	Grade 2	IDC NOS	Grade 2	1
36	643/12	F382/12	56	4cm	Central	DC	Grade 2	IDC NOS	Grade2	0
37	716/12	F450/12	40	4.5cm	Outer	DC	Grade 2	IDC NOS	Grade 2	9
38	717/12	F399/12	50	3.5cm	central	DC	Grade 2	IDC NOS	Grade 2	0
39	774/12	F393/12	50	5.5cm	central	DC	Grade 3	IDC NOS	Grade 3	5
40	775/12	F2085/12	65	4cm	Inner	DC	Grade 1	IDC NOS	Grade 1	0
41	830/12	F446/12	50	4cm	central	DC	Grade 1	IDC NOS	Grade 1	0
42	848/12	F54/12	70	4cm	central	DC	Grade 3	IDC NOS	Grade 1	0
43	849/12	F530/12	86	4.5cm	central	DC	Grade 2	IDC NOS	Grade 2	0
44	850/12	F182/12	56	3.5cm	Outer	DC	Grade 2	IDC NOS	Grade2	0
45	851/12	F54/12	50	4.5cm	Outer	DC	Grade 3	IDC NOS	Grade 3	0
46	906/12	F506/12	37	9.5cm	Inner	DC	Grade 2	IDC NOS	Grade 2	2
47	1136/12	F648/12	42	5cm	Outer	DC	Grade 3	IDC NOS	Grade 3	7

48	1147/12	F703/12	42	4cm	Inner	DC	Grade 2	IDC NOS	Grade2	2
49	1204/12	F757/12	39	6cm	Outer	DC	Grade 1	IDC NOS	Grade 1	3
50	1211/12	F846/12	54	4cm	Inner	DC	Grade 2	IDC NOS	Grade 2	3

KEY TO MASTER CHART

- IDC NOS-Invasive duct carcinoma no special type
- DC-Ductal carcinoma

ABSTRACT

Fine needle aspiration cytology is the diagnostic tool used in the early detection of breast cancer.It is a valuable method to assess the behavior of the tumor while it is still in vivo. However cytological grading system is not well established as like histological grading.In the present study, fifty cases of Invasive duct carcinoma including certain special types like mucinous carcinoma, papillary carcinoma and atypical medullary carcinoma were analyzed. Cytological smears were graded using Robinson grading system and correlated with the well known histological grading as per Scarff bloom Richardson grading system .Cytological grading system was also correlated with other prognostic factors like age, tumor size and lymph node status. This grading system predicts the prognosis from fine needle aspirates and helps the surgeon to plan for further treatment.

KEY WORDS:Invasive duct carcinoma NOS,FNAC,Robinson grading system